UFAW HANDBOOK ON THE CARE & MANAGEMENT OF LABORATORY ANIMALS

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THE U F A W HANDBOOK ON THE CARE AND MANAGEMENT OF LABORATORY ANIMALS

With an Appendix on Statistical Analysis

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FOREWORD

EVERY scientist who correctly appreciates the part played by laboratory animals in research work recognizes the importance of using the right technique in their maintenance and handling. Not only is the accuracy of our results at stake, but we are under an obligation to show the utmost consideration for the animals themselves. It behoves us, therefore, to see that they are produced and maintained under conditions that will conform to a high standard of humaneness and will at the same time ensure that our conclusions shall be thoroughly reliable.

Many of us have attempted to improve on the older methods of rearing and keeping laboratory animals and doubtless in some cases our efforts have been successful. The information has, however, been largely confined to the place in which the knowledge was gained, and only exceptionally has it been available to other laboratories.

In this handbook there is brought together a mass of information which will satisfy a long-felt want. It provides a collection of knowledge gained by first-hand experience, and even the most knowledgeable among us will find in it something which will be of value to him. At any time we may have to make use of species with which we have hitherto been but poorly acquainted, and we now have a reference book which will guide us in this important subject.

The Universities Federation for Animal Welfare is to be congratulated on its choice of writers, and the collaborators on the able manner in which they have presented their subjects.

T. DALLING.

WEYBRIDGE.

PREFACE

This handbook, which has been produced by the Universities Federation for Animal Welfare (U F A W, 284 Regent's Park Road, London, N.3), is intended as a practical introduction to the husbandry of laboratory animals, for use both by research workers and by technicians. It is not to be regarded as a manual of experimental technique.

The method adopted in its compilation has been to secure, wherever possible, an expert worker to draft the chapter on each subject, species or group of species, and to obtain supplementary information by means of a questionnaire. This questionnaire was sent to all holders of a British licence for animal experimentation and to a number of persons and bodies in the United States of America, the Soviet Union, Canada, India, South Africa, Palestine, Sweden, Algeria and elsewhere. U F A W is indebted to various scientific journals (including *Science, Lancet, British Medical Journal, Veterinary Record, Journal of the American Medical Association, Canadian Medical Association Journal* and *Canadian Journal of Comparative Medicine*), to the British Council (through Mrs. E. M. Malley, of the Science Department), to its late President, Sir Peter Chalmers Mitchell, F.R.S., and to Dr. Leptesa (of the Soviet Red Cross and Red Crescent) for their kind assistance in the search for information.

The war interfered, in some cases perhaps rather seriously, with the carrying out of this plan. Many experts were unable to undertake the drafting of chapters or even the provision of information, while a few potential contributors, including Lieut. N. A. W. Hayward, R.M., of Exeter, were killed in action. Death has ended also the participation of contributors in civilian life. The late Mr. R. M. Ranson, of the Bureau of Animal Population, Oxford, had undertaken to draft three chapters and to assist in many other ways, while Miss Phyllis Kelway, of Huddersfield, completed the preliminary draft, upon which Chapter 7 was to have been based, only two days before she died. The late Mr. D. L. Robarts, of Weybridge, had fortunately submitted his contribution to Chapter 4 before his untimely death.

Certain species have been omitted deliberately, except for the brief notes and references in Chapter 23. It was felt that little useful purpose would be served by attempting to deal in the limited space available with the monkey, dog, cat, horse and other ungulates as laboratory animals, and invertebrates also have been but scantily noticed. In a later publication it might be possible to extend the list of species, but for present purposes information of the type given in Chapter 23 is regarded as more helpful in connection with them.

An index has been provided, although for all practical purposes the contents of the book are clearly listed on pp. xi to xiv. It must be stressed here that references to many further sources of general information are to be found in Section 8 of Chapter 2, pp. 42-45.

Several members of the staff of the National Institute for Medical Research (and in particular Dr. C. H. Andrewes, M.D., F.R.S., and Mr. R. E. Glover, M.A., F.R.C.V.S.) were kind enough to read the first drafts of the various chapters and to make valuable suggestions; in many instances it has been possible to make alterations or additions accordingly. Mr. Charles Elton and

his colleagues at the Bureau of Animal Population, Oxford University, have also afforded considerable assistance in the revision of certain chapters and in tracing Mr. Ranson's notes. Dr. G. Lapage, of the Institute of Animal Pathology, Cambridge University, has helped to handle the Russian contributions. Mr. E. W. Shingfield, of the drawing-office staff, Army Operational Research Group, has re-drawn figs. 15, 17, 22, 24, 38, 45–47 and 64–70. My personal thanks are due also to Dr. F. Jean Vinter and Major C. W. Hume, of U F A W, who have rendered invaluable assistance in editing, and to my father, Dr. C. N. Worden, for preparing most of the manuscript for the press.

ALASTAIR N. WORDEN.

East Barnet,
May 1945.

Note added in proof, August, 1946.

Renewed thanks are due to many of those mentioned above, in particular to members of the staff of the National Institute for Medical Research and of the Bureau of Animal Population, Oxford. It has not been possible to take advantage of many kind offers to read the proofs but I am most grateful to Mr. S. J. Edwards (Agricultural Research Council Field Station, Compton), Dr. C. Horton-Smith (Ministry of Agriculture, Veterinary Laboratory, Weybridge) and Dr. J. R. M. Innes (Imperial Chemical Pharmaceuticals), for their assistance in this respect and to my secretary, Miss M. Joan D. Addyman, who has shared in all the tasks at this stage. The following include those who have helped in many ways but to whom adequate acknowledgement or reference is not made elsewhere in the book :—

Prof. E. D. Adrian (Physiological Laboratory, Cambridge), Dr. G. Alexander (Department of Surgical Neurology, Edinburgh), Mr. F. D. Asplin (Ministry of Agriculture, Veterinary Laboratory, Weybridge), Prof. F. G. Bartlett (Psychological Laboratory, Cambridge), Dr. A. Beck (Public Health Laboratory, Burnley), Dr. S. Beck (Glasgow Royal Cancer Hospital), Dr. D. B. Blacklock (Liverpool School of Tropical Medicine), Major J. A. Boycott (R.A.M.C.), Mr. A. Brownlee (Agricultural Research Council Field Station, Compton), Messrs. Warren E. Buck and E. S. Ward (Meems Bros, and Ward, New York), Prof. J. H. Burn (Department of Pharmacology, Oxford), Dr. H. Burrows (Chester Beatty Research Institute, London), Prof. E. P. Cathcart (Physiological Department, Glasgow), Prof. G. A. Clark (Faculty of Medicine, Sheffield), Miss A. S. Cloe (King's College, London), Dr. T. V. Cooper (County Pathologist, Dorchester), Prof. D. P. Cuthbertson (now at the Rowett Research Institute, Aberdeenshire), Sir Henry H. Dale, P.R.S. (Royal Institution, London), Sir Jack C. Drummond (now Scientific Director, Boot's, Nottingham), Prof. A. N. Drury (Lister Institute of Preventive Medicine, London), Dr. F. J. Dyer (College of the Pharmaceutical Society, London), Dr. I. S. Farrel, Dr. J. S. Faulds (Cumberland Pathological Laboratory, Carlisle), Mr. D. A. Finlayson (Ogilvie Flour Mills Co., Winnipeg), Mr. J. R. Finlayson, Dr. Myron Gordon (New York Zoological Society), Mr. R. F. Gordon (Ministry of Agriculture, Veterinary Laboratory, Weybridge), Dr. W. S. Gordon (Agricultural Research Council Field Station, Compton), Prof. James Gray (Zoological Laboratory, Cambridge), Dr, A. Haddow (Chester Beatty Research Institute, London), Prof. J. B. S. Haldane (University College, London), Dr. John Hammond (School of Agriculture, Cambridge), Dr. M. F. Harbord (Howmill, Carlisle), Dr. Evelyn Hewer (London School of Medicine for Women), Prof. F. G. Hitch (Royal Naval Medical School, Greenwich), Dr. F. D. M. Hocking (Royal Cornwall Infirmary, Truro), Prof. A. StG. Huggett (St. Mary's Hospital, London), Mr. W. T. Hunt (Department of Physiology, Edinburgh), Miss Ina Jones-Allen (Department of Animal Health, Aberystwyth), Prof. E. L. Kennaway (Chester Beatty Research Institute, London), Dr. E. H. Koerner (Royal Herbert Hospital, London), Dr. A. J. Leigh (Ruthin Castle, North Wales), Prof. R. T. Leiper (London School of Hygiene and Tropical Medicine), Dr. J. T. Lewis (Universidad Nacional del Litoral, Argentina), Mr. H. W. Ling (Department of Pharmacology, Oxford), Dr. E. C. Lowe, (Southport Infirmary), S/Ldr. B. J. B. Lucas

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Sincere apologies are offered for any omissions in this list and for failure to take advantage of all the kind offers to assist in the preparation of the handbook.

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CHAPTER 1

LAW AND PRACTICE: THE RIGHTS OF LABORATORY ANIMALS

By Major C. W. Hume, M.C., B.Sc.

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1. Historical Note

The British Association and British Medical Association. At its Liverpool meeting, 1870, the British Association for the Advancement of Science requested the Committee of Section D:

> to consider from time to time whether any steps can be taken by them, or by the Association, which will tend to reduce to its minimum the suffering entailed by legitimate physiological enquiries; or any which will have the effect of employing the influence of this Association in the discouragement of experiments which are not clearly legitimate on live animals.

At the Edinburgh meeting in the following year a committee appointed by Section D presented the following report, which was adopted by the General Committee:

(i) No experiment which can be performed under the influence of an anæsthetic ought to be done without it.

(ii) No painful experiment is justifiable for the mere purpose of illustrating a law or fact already demonstrated; in other words, experimentation without the employment of anæsthetics is not a fitting exhibition for teaching purposes.

(iii) Whenever, for the investigation of new truth, it is necessary to make a painful experiment, every effort should be made to ensure success, in order that the suffering inflicted may not be wasted. For this reason, no painful experiment ought to be performed by an unskilled person with insufficient instruments and assistance, or in places not suitable to the purpose, that is to say, anywhere except in physiological and pathological laboratories, under proper regulations.

(iv) In the scientific preparation for veterinary practice, operations ought not to be performed upon living animals for the mere purpose of obtaining greater operative dexterity.

Signed: *M. A. Lawson*, Oxford. *G. M. Humphry*, Cambridge.
John H. Balfour, Arthur Gamgee, Edinburgh.
William Flower, Royal College of Surgeons, London.
J. Burdon Sanderson, London.
George Rolleston, Secretary, Oxford.

In 1871 a Committee of the British Medical Association reported in the same ense. Although it is now agreed that these recommendations did not go far

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enough, nevertheless when we remember that they represent the first informed attempt to arbitrate between the claims of science on one hand and the rights of animals on the other, that the signatories were parties to the suit, and that Britain had only recently discarded such things as colonial slavery and the sweating of children, we must pay homage to the breadth of mind and humanity of the men of science who laid such a foundation for the subsequent developments which will now be described.

A petition calling for legislation was signed by Darwin, Huxley, Jenner, Owen, the Presidents of the Royal Colleges of Physicians and Surgeons, and other leaders of scientific thought, but on 14 April, 1875, Darwin wrote to Hooker: " We now think it advisable to go further than a mere petition." A Bill was accordingly drafted by his son-in-law, R. B. Litchfield, and entrusted to Lyon Playfair, F.R.S., afterwards Lord Playfair. The possessor of this apt patronymic was M.P. for the Universities of Edinburgh and St. Andrews, had held a Chair of Chemistry in Edinburgh, as Gladstone's Postmaster-General had introduced halfpenny postcards, had invented the incendiary shell and the gas shell, and as a Commissioner of the Great Exhibition had helped to found the Royal College of Science.

It would be unfair not to mention that attention had first been called to the subject by a lay movement organized by Miss Frances Power Cobbe, with encouragement from Queen Victoria, F.R.S., Froude, Tennyson, Carlyle Lecky, Martineau, Lord Shaftesbury, Cardinal Manning, the Lord Chief Justice, the Lord Chief Baron, and others. The anti-vivisectionists were inspired by humane feelings that deserved respect, but the methodology of scientific research lay beyond their comprehension and they rested their case on an extraordinary claim to the effect that animal experimentation must be useless, though Miss Cobbe herself wisely protested against their doing so (*Life*, vol. 2, p. 292). Moreover they refused to discriminate between callous and humane research workers, and held all alike up to execration as monsters of cruelty. In this way they alienated sympathizers, and even Darwin, who was too humane to fish with live worms and had written to Ray Lankester (22 May 1871) that vivisection was a subject that " made him sick with horror " and kept him awake at night, felt compelled to publish a rebuttal of the anti-vivisectionists' sweeping allegations. He wrote to Romanes (22 April, 1881) " I thought it fair to bear my share of the abuse poured in so atrocious a manner on all physiologists." Tempers were lost on both sides, and the discussion degenerated into a dogfight. In fact, the dogs have gone on barking and scuffling for three-quarters of a century with all the zest and gusto that those engaging animals do bring to that sort of employment. One result is that animal protectionists as such have no voice in implementing the legal rights that have been accorded to laboratory animals. Another is that the constructive discussion of Home Office practice, *sine ira ac studio*, cannot be carried on in public. Another is that public opinion in scientific circles, which in the eighteen-seventies befriended animals as a matter of course, has until recently been rendered allergic to any reference to the prevention of cruelty. Then animals enjoyed the open patronage of scientists as such, but subsequently they were largely deprived of it by the polemical intemperance of those who intended to act as their champions.

The Cardwell Commission. In May 1875 Lyon Playfair introduced his Bill in the Commons, but a week earlier a Bill promoted by anti-vivisectionists had

been introduced in the Lords. On the advice of Disraeli's government both Bills were therefore withdrawn, and a Royal Commission was appointed. The Chairman was that Viscount Cardwell who as Secretary for War had founded the Army Reserve. The other members were T. H. Huxley, P.R.S., some time Hunterian Professor at the Royal College of Surgeons, and Fullerian Professor of Physiology at the Royal Institution ; Sir Eric Erichsen, F.R.S., some time Professor of Surgery at University College, London ; W. E. Forster, who had been the minister responsible for the Elementary Education Act of 1870 ; Lord Winmarleigh, formerly Chief Secretary for Ireland ; Sir J. B. Karslake, who had held office as Solicitor-General and Attorney-General ; and R. H. Hutton, who had been Professor of Mathematics at Bedford College, London, and joint editor of the *National Review*, the *Economist* and the *Spectator*.

The main report of this weighty Commission was unanimous, but Mr. Hutton added a minority report to the effect that certain species which are popular in this country should be exempted from experiment. The main report stated, *inter alia* :

> that the infliction of severe and protracted agony is in any case to be avoided ; that the abuse of the practice (of vivisection) by inhuman or unskilful persons . . . is justly abhorrent to the moral sense of Your Majesty's subjects generally, not least so of the most distinguished physiologists and most eminent physicians and surgeons.

With reference to the infliction of any pain that was not absolutely necessary they referred to Charles Darwin's dictum that " it deserves detestation and abhorrence " and added :

> This principle is accepted generally by the very highly educated men whose lives are devoted to scientific investigation and education. . . .

On the other hand, Huxley, Erichsen and their five colleagues unanimously recorded the following opinion :

> Besides the cases in which inhumanity exists, we are satisfied that there are others in which carelessness and indifference prevail to an extent sufficient to form a ground for legislative interference. . . . Cases may not improbably arise in future in which the physiologist may be disposed to underrate the pain inflicted. . . .

The Commission's proposals for legislation were consistent with the British Association's report, and are embodied in the present statute with minor changes and provision for administrative machinery. The concluding paragraph of the unanimous report of the Cardwell Commission was as follows :

> We believe that by such a measure as we have now proposed the progress of medical knowledge may be made compatible with the just requirements of humanity. In zeal for physiology the country of Harvey, Hunter, Bell and Darwin may well endure the test of comparison. We trust that Your Majesty's Government and the Parliament of this Kingdom will recognize the claim of the lower animals to be treated with humane consideration, and will establish the right of the community to be assured that this claim shall not be forgotten amid the triumphs of advancing science.

The Act of 1876. The ensuing Government Bill, which became the statute that is in force to-day, was introduced in the Lords by the Earl of Caernarvon on 15 May, 1876. In its original form it appears to have been drafted without sufficient knowledge of the way in which research is done, but it was amended in Committee of the Lords to meet the objections raised on behalf of science. In moving the second reading in the Commons on 9 August (Hansard, vol. 231) :

> Mr. Assheton Cross (Home Secretary) said [col. 890] that they were endeavouring to put in practice and carry out simply the resolutions of the British Association ; and [col. 894] that the Bill, if passed, would set an example to the world that the medical and scientific men of this country had put down the infliction of unnecessary cruelty and pain under the guise of scientific enquiry.

The Bill was attacked on behalf of the anti-vivisectionists by Mr. Holt, and in defence of the reputation of the medical profession by Dr. Ward and by Robert Lowe, F.R.S. The latter, afterwards Lord Sherbrooke, was one of Bright's Adullamites, the first M.P. for the University of London, and an ardent advocate of bicycling.

> Mr. Lowe (University of London) said the language of the Bill " was such as to lead to the supposition that the persons against whom it was directed were unworthy of trust and had wicked propensities." He then turned to the general law against cruelty to animals and pointed out that " the law did not say a word in favour of non-domestic animals ; so far as they were concerned, their charter of freedom and mercy had not yet begun to be written in the legislation of this country." (Later, in the Committee stage of the Bill, he was to move, without success, the addition of a clause dealing comprehensively with all forms of cruelty and imposing severe penalties on anybody who should " cruelly abuse or torture *any* animal.")

Sir John Lubbock also condemned, by contrast with " the general sentiments of English men of science, the dreadful sufferings of animals which are caught in traps, and which have so often been known in their torment to gnaw off the limb by which they were held fast." Honour having been satisfied, all parties agreed to the second reading, which took place without a division after a significant intervention by Lyon Playfair.

> Mr. Lyon Playfair (Universities of Edinburgh and St. Andrews) said [col. 921] : " We propose to regulate one minute corner of the vast field of the cruelty of the world. When we do so we are bound to survey the whole field for legislation on a future occasion. . . . I know of nothing more cruel than the mode of supplying rabbits to the Billingsgate market. They are caught in traps, and struggle through the whole of the night in cruel anguish, often with broken legs. . . . Now if one of our dozen physiologists was to describe a single experiment made with such reckless disregard of animal suffering he would be scouted by his brother physiologists and driven out of the Kingdom.
>
> " There are, however, well-grounded reasons for legislation, and these operated upon me last year when I introduced a Bill on the same subject. . . . It was in reality prepared by very eminent physiologists, among whom I may mention Mr. Darwin, Mr. Huxley, and Dr. Burdon Sanderson,

and was approved by the heads of the two Colleges in London. It is obvious, therefore, that physiologists themselves were very early in the field in recognition of the necessity for legislation. . . .

"A statute cannot regulate individual motives and consciences when these have been placed in antagonism to its provisions ; but a statute which asks physiologists to co-operate with the State in preventing cruelty to animals will be obeyed with alacrity."

The Ram Commission. The statute conferred wide discretionary powers on the Home Secretary, leaving him free to develop an administrative policy in the light of experience, and thirty years later Campbell Bannerman's government appointed a new Royal Commission to examine the results. It sat for six years and put 21,761 questions to 60 witnesses ; two of its members, including the original Chairman, Viscount Selby, failed to survive. The eventual Chairman was A. J. Ram, K.C., Recorder of Wolverhampton, and five of the remaining seven members were medical men, namely Sir J. McFadyean, who was also a veterinarian and Principal of the Royal Veterinary College ; Sir W. S. Church, President of the Royal College of Surgeons ; W. H. Gaskell, F.R.S., University Lecturer in Physiology, Cambridge; Sir William Job Collins, F.R.C.S., M.P. for the University of London and some time Chairman of the London County Council ; and Dr. George Wilson. The other two members were Col. A. M. Lockwood, C.V.O., Coldstream Guards, Retd., afterwards Lord Lambourne, Lord Lieutenant of Essex, for many years Chairman of the Kitchen Committee of the House of Commons, a horse-and-dog-loving squire and a Vice-President of the R.S.P.C.A. ; and Sir M. D. Chalmers, formerly Parliamentary Counsel at the Treasury. All the commissioners signed the report, but three of them appended reservations which will be noticed later.

After examining the charges brought by anti-vivisectionist witnesses against the working of the Act, the Commission said :

> So far as we can judge we believe that holders of licences and certificates, with rare exceptions, have endeavoured with loyalty and good faith to conform to the provisions of the law.

On the other hand they referred to two physiologists who had expressed views which showed them to be blind to humane requirements, and added the following litotes :

> to grant a licence or certificates to any person holding such views . . . is calculated to create serious misgivings in the minds of the public.

As regards the utility of experiments on animals they found that "notwithstanding failures, valuable knowledge has been acquired." With regard to anæsthetics they found that

> it is possible to establish and maintain, even for long periods of time, a condition of complete insensibility to pain in the lower animals, as in man, yet their administration in order to secure such anæsthesia requires caution and watchfulness as well as the natural solicitude of a humane and skilful operator.

With regard to the pithing of frogs and other animals they found that

> no lesser operation than a complete destruction of the brain (not only of

> the cerebral hemispheres but also of the basal ganglia) or decapitation should be accepted as equivalent to the production of complete anæsthesia. . . . The operation of pithing a warm-blooded animal should be conducted only by a licensed person, and the operation itself should be performed under an adequate anæsthetic.

The Commission recommended that the number of Inspectors should be increased, that research should be centralized as far as possible, that curare should not be used at all unless an inspector is present, that the responsibility of the Home Secretary should be in all respects maintained, that licences and certificates should continue to be withheld from foreigners except for work under supervision, that antiseptic and pain conditions should continue to be attached to licences, that in some cases the Home Secretary should call for an immediate or special record or report, and that the Home Secretary should appoint a body of advisers who should not themselves hold licences.

The most radical recommendation made by the Ram Commission related, however, to the pain condition attached to licences. Previously the pain condition had applied only "if the main result of the experiment had been attained."

Dealing with the notion that any means that is indispensable to the advance of scientific knowledge would be justified by the end in view, the Commission remarked:

> The quest for knowledge may, of course, conflict with moral principle. Thus torture, once largely employed as an instrument for obtaining judicial evidence, is a case in point. The practice, though justified by Bacon, was discarded in England in the seventeenth century and in Scotland a little later, but was continued on the Continent to the close of the eighteenth century. Here the infliction of human suffering in view of contemporary morals was not only regarded as unobjectionable in the pursuit of knowledge but was purposely utilized to extract information. The growth of the average moral sense of mankind has condemned and abolished the practice, not because it did not lead to useful knowledge, but because, however useful the result, the means could no longer be justified.

The Commission now recommended that Inspectors should be empowered to order the killing of an animal and that the Home Secretary should require

> that in all cases in which in the opinion of the experimenter the animal is suffering severe pain which is likely to endure, it shall be his duty to cause its painless death, *even though the object of the experiment has not been attained.*

Further than that they felt unable to go, partly because

> public opinion sanctions the infliction of pain which is not only severe but of long duration . . . in the destruction of rabbits and of rats and other vermin by traps and painful poisons.

However, three of the eight commissioners (Collins, Lockwood and Wilson), while agreeing that "experiments adequately safeguarded by law faithfully administered are justified and should not be prohibited," urged in a reservation memorandum that

> if in the course of scientific investigation the pursuit of knowledge leads to the infliction of real or obvious suffering it is right that the pursuit should cease rather than that the infliction of suffering should continue.

That is to say, they wished to remove from the pain condition the proviso that the pain should be "likely to endure." One of them (Wilson) added a further memorandum in which he expressed a critical attitude towards research.

2. The Law and its Application

The nature and scope of the statute. The Cruelty to Animals Act, 1876, is not an infliction devised by cranks for making biologists fill up forms. It is a contract laboriously hammered out by some of the ablest scientists and administrators of their day with two objects: to define limits for the sacrifice which the public is prepared to exact from animals as the price of medical knowledge, and to protect research workers against vexatious litigation and obloquy. The good faith as well as the humanity of scientists is engaged, for obviously it is out of the question for the Home Office Inspectors to be constantly policing every laboratory; to visit each establishment two or three times a year is the most they could hope to accomplish. On the other hand, the Home Office publishes a very reassuring annual statement to ease the public mind, and does so in the faith that research workers are conscientiously observing the terms on which their licences are granted. Hence the importance of having clearly in mind what those terms are, an achievement which the structure of the Act does little to facilitate.

Section 3 of the Act lays down restrictions so severe that they would rule out research in nutrition and bacteriology, for instance, and then empowers the Home Secretary (who is the "Secretary of State" referred to in the Act) to relax certain of the restrictions which it has laid down. The effect is to put upon the Home Secretary the responsibility for determining how the objects of the Act are to be achieved, and for this purpose it gives him very wide discretionary powers.

The Act relates (sec. 2) to experiments on living animals which are "calculated to give pain." The Home Secretary, to avoid ambiguity and in exercise of his statutory powers, construes this phrase to mean

> any procedure calculated to interfere with the normal health and comfort of an animal.*

The law is thus applicable to a wide range of research, and for practical convenience even control animals are counted in the official statistics.

The Act forbids, and the Home Secretary is not empowered to permit, any experiments except those which are

> performed with a view to the advancement by new discovery of physiological knowledge or of knowledge which will be useful for saving or prolonging life or alleviating suffering.

The Parliamentary debates on the original Bill make it clear that the term "physiology" covers all that would now be called "animal biology." The

* Recently he has removed certain pregnancy tests from the ambit of this definition.

permissible subject-matter includes, therefore, any branch of pure animal biology, but as regards applied biology only medical and veterinary researches are permitted. The Home Secretary could not legally license painful experiments having a commercial objective. The expression "new discovery" precludes the repetition of experiments when once their result has been established, but proviso (4) of section 3 provides for the relaxation of this restriction "for the purpose of testing a particular former discovery alleged to have been made" if certificate D is furnished (p. 9) to the effect that such testing is absolutely necessary.

The only persons who are permitted to carry out the experiments envisaged by the Act are those who are licensed by the Home Secretary, subject to conditions which he is empowered to impose. If any unlicensed person carries out such experiments he exposes himself to heavy penalties, and experimentation by technically incompetent amateurs and undergraduates, which occurred before the passing of the Act, appears to have been effectively stamped out. The rôle of the laboratory assistant is restricted by the following notice which appears on the licence :

> N.B.—The Licensee is warned that he may not delegate his authority to any other person to perform in whole or in part the experiments covered by this Licence or by any Certificate under the Act.

A licence is a protection against prosecution under the Protection of Animals Act, 1911, and by section 21 a licensee cannot be prosecuted under the Act of 1876 without the written assent of the Home Secretary ; this is his *quid pro quo* for having to submit to official regulation and control.

Experiments must be performed in a registered place named in the licence ; this is a condition imposed by the Home Secretary under a power conferred on him by section 7.

In no circumstances may experiments be performed on living animals for the purpose of attaining manual skill (sec. 3 (6)).

The Act unfortunately does not apply (sec. 22) to invertebrate animals, though it does apply to cold-blooded vertebrates. Nevertheless it is to be believed that humane biologists will give invertebrates the benefit of the doubt even if they question the sensibility of these creatures to any given stimulus ; see p. 18.

Administration. The Home Secretary has absolute power to grant or withhold a licence, subject to any conditions he may choose to attach to it. In practice he always does attach certain conditions which will be mentioned later.

An application for a licence must be recommended in writing by two persons of whom the first must be the President of one or other of certain learned institutions which are specified in section 11 and include, for instance, the Royal Society, the Royal Colleges of Surgeons and of Physicians, and the Royal College of Veterinary Surgeons. The second signatory must be a university professor of physiology, medicine, anatomy, medical jurisprudence, materia medica or surgery. The applicant is required to state the object of his research, the general nature of the proposed experiments, the place in which he wishes to perform them, and the nature of any certificates of exemption which he intends to submit. The licence is granted for a specified period but is renewable. The number of animals to be used may be specified but

usually is not, unless the experiment is of a particularly serious nature. The applicant is not permitted to practise until he has been notified in writing that licence and certificates have been allowed by the Home Office.

Experiment under licence alone is subject to very stringent restrictions, but certain of these, which will be detailed later, may be relaxed (secs. 3, 11) subject to the conditions that : (1) a certificate to the effect that such relaxation is absolutely necessary, or that without it the object of the experiment would be frustrated, must be signed by the persons who are authorized to endorse applications for licences ; (2) a copy of the certificate must be forwarded to the Home Secretary ; (3) a certificate must not be acted upon until the licensee has been notified in writing that it has not been disallowed by the Home Secretary ; and (4) the Home Secretary may at any time disallow or suspend the certificate (sec. 11).

The Home Secretary exercises his powers through Inspectors appointed under section 10. It is their business not only to visit laboratories but also to scrutinize applications for licences and copies of certificates, to check records and reports of work done under licence, to ensure that the licensees make the returns that are required of them, and generally to see that the law is complied with and to implement decisions on policy.

In deciding whether to grant a licence or allow a certificate the Inspectors appear to be guided mainly by precedent, but in appropriate circumstances, as in the case of a novel experimental procedure or one likely to arouse serious criticism, they may refer for guidance to an Advisory Committee set up by the Home Secretary in accordance with the Ram report and consisting of eminent representatives of medicine and surgery, with a judge of the High Court as Chairman. The Committee's powers are advisory only, and the responsibility for final decisions rests on the Home Secretary.

The conditions attached to a licence require that a licensee shall keep a written record of all his experiments, and it must be open to examination by the Inspector at any time. He must as soon as is practicable send to the Home Office the fullest published accounts of his work, indicating its place and date in a covering letter (further condition 5). At the end of the year he must report the number and nature of all experiments performed, and he may be called upon for special interim reports (further condition 4).

Experiments involving operations. Although to-day only 4 per cent of British experiments belong to this class, in 1870 physiology was the dominant branch of experimental biology and the typical experiment did involve an operation ; hence the term "vivisection," and also the enactment that, in experiments performed under licence alone (sec. 3 (3, 4)),

> the animal must during the whole of the experiment be under the influence of some anæsthetic of sufficient power to prevent the animal feeling pain ; and the animal must, if the pain is likely to continue after the effect of the anæsthetic has ceased, or if any serious injury has been inflicted on the animal, be killed before it recovers from the influence of the anæsthetic.

On the other hand, in some 80 per cent of operational experiments the operation is merely preliminary to subsequent observations ; it may comprise, for instance, the excision of the gonads or other glands. In such cases the animal is permitted to recover from the anæsthetic after an operation, but

before this may be done certificate B must not only be furnished (p. 9), but also be allowed in writing by the Home Office. It is to the effect that :

> the killing of the animal before it recovers from the influence of the anæsthetic administered to it would necessarily frustrate the object of the experiment.

Science is more advanced in this matter than industry. The Animals Anæsthetics Act, 1919, permits such operations to be done without anæsthetics on pigs and sheep, although the object of the operations is to increase commercial profit.

As regards anæsthesia, section 4 lays down that :

> the substance known as urari or curare shall not for the purposes of this Act be deemed to be an anæsthetic

and one of the conditions attached to every licence is that :

> no experiment in which curare is used shall be performed without the special permission of the Secretary of State ; and 48 hours' notice . . . shall be given to the Inspector of the District.

This last condition does not apply to decerebrated animals in which the cerebral hemispheres *and basal ganglia* have been destroyed, but it does apply in every other case on the ground that the normal tests for anæsthesia are invalidated by the disabling of the motor nerves by curare. The same principles must be held to apply, by analogy, to any other technique in which there is a risk of disabling the motor nerves while leaving the sensory system operative ; for instance to the " nightmare state " observed by Leduc and by J. Hertz (*Revue de Pathologie Comparée*, 1933, p. 385) when an electric current of suitable strength in relation to its wave-form was passed through the head.

In surgical practice it has been found necessary for the surgeon to be assisted by a separate anæsthetist, and *a fortiori* during an operation on any animal an assistant ought to give his whole attention to maintaining anæsthesia, unless the anæsthetic is of such a nature as to render this precaution superfluous.

The Home Office reports annually that "in no case has a certificate dispensing entirely with the use of anæsthetics been allowed for an operative procedure more severe than subcutaneous venesection." No doubt the word " operation " was first applied to cutting operations, but in order that the impression conveyed by the report may be valid all such interferences as bruising, crushing, burning and blast from bombs must clearly be reckoned as operative procedures.

When, under certificate B, an animal is to be allowed to recover consciousness after an operation, the following conditions are attached to the licence :

> The animals shall be treated with strict antiseptic precautions and if these fail and pain results the animals shall be immediately killed under anæsthetics.
>
> All operative procedures shall be carried out under anæsthetics of sufficient power to prevent the animal from feeling pain.
>
> If at any time an animal is found to be suffering pain . . . (here follow the pain conditions, p. 11).

Relaxation of the antiseptic or aseptic precautions is occasionally permitted ; for instance, in experiments on antiseptics.

Experiments not involving operations. Certain experiments which do not involve mechanical interference may cause as much pain as those which do ; dosage with corrosive poisons and electric tetanization are examples of this class. They are on the same footing as operations and demand similar precautions as regards anæsthesia.

In the great majority of experiments, however, the initial stages at least are substantially painless, and it would not be feasible to maintain anæsthesia throughout. To this class belong most researches in nutrition, bacteriology and the like. In order that anæsthesia may be dispensed with in such cases the licence must be supplemented by certificate A, and the licensee, before he begins work, must have been notified in writing that the Home Office has not disallowed the certificate, which is to the effect that

> insensibility in the animal . . . cannot be produced by anæsthetics without necessarily frustrating the object of the experiment.

Whenever certificate A or certificate B is allowed, the following pain conditions are attached to the licence :

> If an animal at any time . . . is found to be suffering pain which either is severe or is likely to endure, and if the main result of the experiment has been attained, the animal shall forthwith be painlessly killed ;
>
> If an animal at any time . . . is found to be suffering severe pain which is likely to endure, such animal shall forthwith be painlessly killed ;
>
> If an animal appears to an Inspector to be suffering considerable pain, and if such Inspector directs such animal to be destroyed, it shall forthwith be painlessly killed.

As regards the construction to be put upon these conditions, the following remarks have been made by a working physiologist in a letter to the author : " I imagine that the principle is exactly the same as one would apply to a pet animal. If he was in pain the first attempt would be to relieve the pain and to eradicate its source, and only if this proves impossible, i.e. if the pain is likely to endure, is the animal destroyed."

In no circumstances may an experimenter permit suffering which is both severe and likely to endure, *whether or not the main object of the experiment has been attained* ; and when the main object of the experiment *has* been attained, neither brief severe suffering nor prolonged mild suffering may be permitted ; only brief mild suffering is allowed in pursuance of subsidiary objects. The pain rules will be further considered on p. 20 ; they relate to a difficult problem and represent a compromise carefully worked out. In interpreting them the research worker is responsible for deciding whether a given animal is suffering severely, whether the suffering is likely to endure and, when one of these situations exists but not both, whether the main object of the experiment has been attained. He has to do this sincerely, objectively and generously, without being biased by the threat to the success of his experiment, the child of his brain.

Demonstration experiments. " Any exhibition to the public, whether admitted on payment of money or gratuitously, of experiments on living

animals calculated to give pain " is illegal (sec. 6). The courts would probably hold that this applies also to cinematograph films.

Experiments in illustration of lectures are subject to the conditions that : (1) certificate C must be furnished and allowed, to the effect that the demonstration is necessary " for the due instruction of the persons to whom such lectures are given with a view to their acquiring physiological knowledge or knowledge which will be useful to them for saving or prolonging life or alleviating suffering " ; and (2) the animal must be fully anæsthetized throughout.

Privileged animals. Section 5 establishes three grades of privilege among animals. The highest is assigned to horses, asses and mules, which may not be used for any experiment under the Act if any other species will serve ; the second grade is assigned to cats and dogs, which may not be used for an experiment without anæsthetics if any other animal will serve ; and the third grade is assigned to monkeys, rodents and all other vertebrate species, which enjoy the general protection of the Act whereas invertebrates do not. Mr. Lowe M.P. for the University of London, expressed in Committee of the Commons a strong objection to the section, on the ground that it " established an aristocracy of animals. Who were they that they should sit in judgment on these animals, inflicting pain on one and exempting others ? " The reply of the Home Secretary, Mr. Assheton Crosse, was based on two factual assumptions (1) that the privileged species are more intelligent than the other species, and (2) that intelligence and sensibility to pain are closely correlated. These assumptions are untenable ; mules are not more intelligent than rats or monkeys, and it is a gross error to confuse intelligence with sensibility to pain No doubt the English sportsman has a right to favour horses and dogs, the lonely woman cats and parrots, the Hindoo cows, the Bantu ground hornbills the Red Indian beavers, and the present writer rats (people love best those animals which they have learned to understand), but these preferences must not be confused with justice or humaneness, with which they have no connection whatever. Small animals have the advantage, however, that they can more easily be fed and housed in comfort, and there are obvious technical advantages in using fast-breeding species.

If a horse, ass or mule is to be used, certificate F must be furnished to the effect

> that the object of any such experiment will be necessarily frustrated unless it is performed on a horse, ass or mule, and that no other animal is available for such experiment.

If a dog or cat is to be used without anæsthetics under certificate A, then certificate E must be furnished to the effect

> that the object of any such experiment will be frustrated unless it is performed upon an animal similar in constitution to a dog or cat, and that no other animal is available for any such experiment.

If a dog or cat is to be used with anæsthetics and allowed to recover consciousness under certificate B, then certificate EE must be furnished. It is similar in effect to certificate E but has a different preamble.

Sanctions. The penalties laid down for infringement of the Act are £5 for the first offence and £100 or 3 months' imprisonment for any subsequent offence. In practice these penalties are not invoked, and doubtless any

research worker who felt tempted to open defiance of the law would be sufficiently deterred by the prospect of having his licence cancelled.

Short of such an extreme, however, great variations are conceivable in the care, detachment and generosity with which an experimenter applies his judgment to the questions put to him by the pain conditions (p. 11) in the course of his practice. Some aids which are available to him will be mentioned on p. 16, and he will doubtless bear in mind that, apart from the categorical imperative of his own conscience, any justification he might give to anti-vivisectionist allegations would be prejudicial to the cause of research. But the most valuable aid that he can have in the discharge of his obligations is a humane and compassionate public opinion among colleagues of his own and other laboratories, and among men of science generally.

The law overseas. The information available on this subject at the moment of writing is scanty and for the most part some years old. The background against which it must be viewed is the fact that in most parts of the world the standard of humaneness in matters quite unconnected with science has not progressed even as far as it did in early-Victorian Britain, added to the fact that sympathy has been alienated by the undiscriminating character of the anti-vivisectionist propaganda.

In France and Italy there has been no effective regulation of experiments, although Mussolini tried to make a gesture in that direction. The late Prof. E. H. Starling told the writer that in his opinion the scientific value of French and Italian physiology had been gravely impaired by failure to eliminate the disturbing effects of pain. In Russia (1945) there appears to be no law for the protection of laboratory animals. In Japan (Korea, 1937) there was no system of licences; experiments were done by students as well as staff in medical schools under Christian and under Japanese control, although in the latter a Buddhist memorial service was held annually for "those animals which had offered their bodies to medical research." In China (1937) there were, according to the British Consul at Shanghai, no licensing and no laws; animal experiments are understood to have been done by students. No measures for the protection of laboratory animals in Spain and Latin America are known to the writer.

In Holland (1938), on the other hand, experimentation was regulated by a voluntary system in which the Netherlands Society for the Protection of Animals played its part. In Norway (1938) experimenters required licences but were otherwise unrestricted. In several of the British Dominions the governments have power to regulate animal experimentation, though no information has been received as to the development of concrete machinery for implementing the law. In Eire the British Act of 1876 is still on the statute book.

In Germany animals in general had long been better treated than in most other countries, and in 1934 a law was signed by Hitler, Frick and Gürtner requiring that experimenters must hold permits and keep records, must anæsthetize unless this would defeat the object of the experiment or cause more distress than the experimental procedure would do, must not do more than one serious experiment on any one animal, and must practise economy in sampling. Experiments might not be used for teaching unless cinematograph films or the like would not serve. In Sweden (1945) animal experiments may be performed only with a permit from the Swedish Medical Board, and in accordance

with a code of statutory rules. In Belgium (1945) the law is that experiments may be carried out only in a laboratory at or connected with a university under the control of the responsible director, and that the animals must be anæsthetized except in case of necessity.

In the United States of America there is no federal law on the subject though a number of state laws prohibit experimentation in schools for children. In 1908, however, a committee of the American Medical Association drew up an ethical code for the guidance of research workers. Medical schools, research institutes and health laboratories are expected to exhibit voluntarily copies of this code, which is exemplified in the following rules as posted at the National Institute of Health :

Rules Regarding Animals.

I. Dogs and cats brought to this laboratory and purchased here shall be held at least as long as at the pound, and shall be returned to their owner if claimed and identified.

II. Animals in the laboratory shall receive every consideration for their bodily comfort ; they shall be kindly treated, properly fed, and their surroundings kept in the best possible sanitary condition.

III. No operations on animals shall be made except with the sanction of the Director of the Institute, who holds himself responsible for the importance of the problems studied and for the propriety of the procedure used in the solution of these problems.

IV. In any operation likely to cause more discomfort than that attending anæsthetization the animal shall first be rendered incapable of perceiving pain and shall be maintained in that condition until the operation is ended.

V. At the conclusion of the operation the animal shall be killed painlessly.

Exceptions to this rule will be made only when continuance of the animal's life is necessary to determine the result of the experiment. In that case, the same aseptic precautions shall be observed during the operation and so far as possible the same care shall be taken to minimize discomfort during the convalescence, as in a hospital for human beings.

(Signed) . . . Director, National Institute of Health.

3. Basic Principles

Ethics. A sharp distinction must be drawn between killing and hurting. The reverence for life taught by Buddhists, Jains and others in Asia and recently by Schweitzer in Europe must be judged on its merits, but must not be confused with the totally different principle that impels humane persons to try to eliminate undeserved pain. These two principles often conflict with one another, as when a Hindoo refuses to put a moribund cow out of pain. The present article is concerned solely with preventing or minimizing suffering and assumes that this object should have priority over reverence for life.

Two extreme opinions may be noticed in passing. At one extreme anti-vivisectionists consider that the end cannot justify the means and that it is wrong to sacrifice one creature for the benefit of another without the consent of the former. Provided that such a view be not buttressed by untenable

factual assertions it is entitled to consideration, but it is unlikely to appeal to readers of this handbook.

At the opposite extreme it has been held that might is right and that man is entitled to take every advantage he can get from mere animals, without regard to any rights of theirs. This attitude was expressed by a foreign physiologist who gave evidence before the Cardwell Commission on an occasion when Huxley was absent, whereupon the latter wrote :

> T. H. Huxley to Charles Darwin, 30 October, 1875 : " I have felt it my duty to act as counsel for science, but if what I hear is a correct account of the evidence K. gave I might as well throw up my brief. I am told that he professed the most entire indifference to animal suffering, and said he only gave anæsthetics to keep the animals quiet !
>
> " I declare to you that I did not believe the man lived who was such an unmitigated cynical brute as to profess and act upon such principles, and I would willingly agree to any law which would send him to the treadmill."

Anti-vivisectionists rest their case in large part on an assertion that research workers regard the infliction of pain on experimental animals as a matter of small importance. The present article is based on the contrary supposition. It assumes that research workers in Britain regard any undeserved pain as an evil, but as the less of two evils if a large amount of suffering can be prevented by means of knowledge got by inflicting only a small amount. Public opinion, as interpreted unanimously by the Ram Commission, on which medical science was well represented, has exhibited a certain degree of unselfishness ; recognizing the great advantages to be got from medical research, it has decided to forgo any of them which necessitate more than a certain degree of sacrifice on the part of experimental animals.

There is a common opinion that the infliction of pain on an unpopular species is a less evil than its infliction on a popular species, and a still less evil than its infliction on a human being, but this view appears to be a rationalization of personal bias and caprice. From an objective point of view a given quantum of undeserved pain appears as equally an evil whether the victim be man, dog or rat, and humaneness as the reduction of this evil to a minimum.

Practical rules. Neither the above principle nor any other that may be referred to it can be applied directly to particular cases. One can derive only a general orientation from an abstract principle, for the reason that it is impossible to measure pain in standard units, or to balance the time-integral of one pain against that of another less intense but more prolonged, or to sum pains felt by different individuals or differing in quality, or to forecast the result of an experiment before it has been carried out, or in general to trace a medical treatment uniquely to a single research, as distinct from a complex of knowledge obtained from a body of research. Hence working rules are needed to guide research workers in their day-to-day handling of experimental animals.

Certain obvious rules present no difficulty. No responsible biologist disputes his being under an obligation to avoid wasteful sacrifice of animals, in any research which involves the infliction of suffering, by ensuring that his experiments shall be methodologically sound, with adequate but not excessive sample-size ; or to use all the technical skill, equipment and care that are demanded by the occasion ; or to ensure that the animal house shall be properly

run ; or to prevent pain or discomfort that is not absolutely necessary, however much trouble the precautions may cost.

Again, when an experiment is to be done it ought to be so planned as to ensure technical efficiency, with the use of modern tests of significance. It is no secret that some of the older work has been insufficiently critical.

The licence form warns the licensee that he " may not delegate his authority to any other person to perform in whole or in part the experiments covered by the licence or by any certificate under the Act." This rule limits the functions of the laboratory assistant ; nevertheless the well-being of the animals depends greatly on the latter, and the selection, training and supervision of assistants is consequently of the first importance. If an assistant is afraid that the animals will bite him, or if they are afraid of him and do bite him, it may reasonably be inferred that his vocation lies elsewhere.

The major responsibility for the working of the Act devolves in practice upon the professor who signs a certificate or an application for a licence. It depends on him that the licensee shall be not only technically competent but also sufficiently humane to be fit for the responsibility of experimenting on animals. No hesitation need be felt in refusing this privilege to anybody who is inclined to think lightly of the rights of animals, for the experience of the Army Operational Research Group has shown that a man with a gift for biology can do valuable work on any problem that involves statistically related variables ; a promising honours student will not be wasted, therefore, if his energies are diverted from responsibilities for which he is not morally fitted and are employed instead on social or anthropological investigations.

The responsibility for observing the pain conditions and other terms on which licences are granted devolves similarly upon experimenters themselves or the heads of their departments. For their guidance in interpreting those terms they can refer to the Inspectors in cases of doubt, and they have available the annual reports of the Home Office (conveying comforting assurances which they are in honour bound to make good), the printed instructions which accompany the licence forms and certificates, the insight into animal psychology which a biological training can furnish, and their own character as human beings. The following maxim may be found helpful : No man has the right to inflict greater suffering than he would himself be prepared to endure voluntarily for the same experimental objective, in the absence of social reasons to the contrary.

When an experiment involving even a limited amount of suffering is proposed, its technical justification has to be critically considered. As a specially important finding in this connection the following extract from the preface to Sir Thomas Lewis's *Pain*, which indicates that animals are not technically suitable subjects for research on pain, will repay study :

> " We have no knowledge of pain beyond that derived from human experience ; yet we may judge of its presence in animals by bodily reactions that human experience has brought us to recognize as its frequent accompaniments, or by the use of stimuli that similar experience tells us should be painful.
>
> " Often, physiological investigation is more profitably undertaken on animals ; in the case of pain, this is not so. Strictly speaking, there are no reliable and usable indices of pain in animals ; there are only phenomena

recognized as frequent associations of pain, such as raised blood pressure, movements of withdrawal or defence, dilatation of pupil, increased respiratory depth, and cries. But some of these are manifestly spinal cord or medullary reflexes and can happen in the absence of pain; and some probably are quite independent of pain, in the sense that the initiating impulses travel by paths other than the paths of pain. Even prolonged struggling and cries are no reliable criteria since they may come, not from pain, but from apprehension; and these are the least usable, for they are associated with the infliction of suffering. Sherrington wrote: 'In all this experimental work on animals the observer has to work through signs of subjective states incomparably inferior in most instances to the verbal communication establishable with an intelligent human being.' Our knowledge of pain, of its sources, of the nerve paths conveying it, has been built up, and will continue to be built up, chiefly by observations upon man. It is built up by tests of the sentient surface in the normal subject, tests of skin deprived deliberately or accidentally of its nerves, the innumerable probings of disease itself, stimulation of deep-lying structures during surgical operations, and ingenious forms of exploration of deep structure from without; all these sources have contributed so abundantly that I shall but rarely need to refer to evidence derived from animals. There is manifest wisdom in seeking knowledge from the direct, rather than from the indirect, source, and here the direct source is prolific; there is, too, the deep satisfaction of knowing that any evidence won from man is indubitably applicable to the human problem."

Sensibility to pain is commonly confused with intelligence in the popular ınd, although a moment's reflection will show that the mistake is a stupid e. There does not even appear to be any tangible evidence of positive rrelation between sensibility and intelligence, beyond the fact that both are ɔdes of consciousness. The only direct evidence on the subject, such as it is, nsists in an adult's comparing his own sensibility with that which he remem-rs himself to have been endowed with in childhood. This test suggests that was more sensitive to pain when his intelligence was in a more primitive age of development, a conclusion supported by the fact that the pain reshold has been found experimentally to rise with increasing age. Two her considerations point in the same direction, albeit a little vaguely. Teleo-gically pain is associated with learning; it teaches the organism to avoid structive stimuli. It may well be the case that the less the intelligence the ore severe is the lesson required. Again, pain sensations may be expected to ılk larger in a simple field of consciousness than they do in one more highly veloped, where they have to compete with other mental states and may also more or less repressed; and this common-sense supposition is supported by me neurological evidence, for the cortex appears to play a part in inhibiting in but not necessarily in registering it (*cf.* Lewis, *infra*). The conclusion that sensibility cannot be inferred from lack of intelligence goes against the assump-ons that are conventionally made at the present time, but conventions are not nding if they are not based on evidence, and in any case the animals must be ven the benefit of any doubt.

The plea that animals are not self-conscious and that pain cannot be felt ıen self-consciousness is absent is a sophism that can hardly be taken

seriously. There is no evidence that animals are not self-conscious ; indeed symptoms of jealousy and a competitive spirit suggest the contrary. But i any case, the pain is the sensation, as distinct from any cognition about th sensation.

In man the pain nerve fibres terminate in the optic thalamus, which appear to be his organ for registering sensations of pain (Lewis, *Pain*, 1942, p. 31 Thalami are present in the brains of cold-blooded vertebrates, but it is some times argued that invertebrates must be insensitive in view of the relativ simplicity of their nervous systems. If such simple-minded logic were admis sible, however, it would lead us to infer that insects cannot breathe from th fact that they have no lungs. It seems safer to be guided by the observatio that invertebrates are capable of rudimentary forms of learning. Moreove the reactions to injury of many molluscs and other invertebrates are s congruous with known pain responses that any other interpretation of thei would have to be based on overwhelming evidence before it could be safel adopted. On the other hand, absence of such reactions does not prove absenc of pain, for " many invertebrates do not look after their young and are no social, so that if they feel pain they are not likely to show it. Further, if the do show it the signs may be such that our sense organs do not record them and anyhow the fundamental difference in structure may make it difficult o impossible for them to make signs that appear to us subjectively as signs o pain " (letter from John R. Baker).

A theory to the effect that even vertebrates are unconscious machines wa put forward by Descartes, whose influence on eighteenth-century thought wa widespread and deep. The theory seems to have sprung from his attempt t construct an *a-priori* proof of the immortality of the soul ; at least the impre sion one gets from a comparison of the fifth part of the *Discours de la Méthod* with the second *Méditation Métaphysique* is that Descartes had either to den consciousness to animals, or to grant them immortality, or to abandon h somewhat sophisticated argument. His disciple Malebranche is said to hav amused himself by beating a dog " in order to hear the creaking of the machine Although obsolete in view of what is now known of the biological relatio between man and the lower animals, this queer heirloom bequeathed by th great-grandfather of modern science is still lying about in intellectual lumbe rooms, and it may be well, therefore, to state the following case for the commo sense view.

My own subjective consciousness is the sole source of any conception I ca form of any mental state, painful or otherwise. I cannot inspect another man mind, and when I picture it or converse with him I have to use my ow thoughts and feelings as symbols of his, interpreting his observable reactio in terms of my own. It is impossible to prove that his mind exists outside m imagination, for every attempt to refute metaphysical solipsism will be foun to beg the question ; the justification for a transcendent interpretation my experience is the pragmatic one, that it enables me to control the latte The same interpretation is applied to the behaviour of animals, with th same justification, by trainers, trappers and others who have to deal wi them.

Finally, while human beings can escape from the severer levels of pain b swooning, it seems doubtful whether most animals, if any, can do so.

4. Some Desiderata

The supply of animals. At a conference held on 21 June, 1943, representative experimental biologists appointed a standing committee to consider this question and it is hoped that increased centralization of breeding will eventually result. Apart from the obvious technical advantages of using homogeneous and healthy stocks whose history is known, the following humanitarian advantages would be gained: (1) the breeding establishments could be put under technically competent management; (2) if mercenary motives were eliminated the living conditions of the stock could be improved; (3) the use of homogeneous stock enables a given degree of precision to be attained with relatively few animals; (4) it is thought that animals bred in institutions are less likely to be perturbed by a laboratory environment than those which are accustomed to a private home.

An important memorandum was adopted by the Conference on 24 September, 1945, but has not been published at the date of proof-revision.

Accommodation. Although in the best laboratories the standard of health and physical comfort is high, the amount of space available is generally inadequate. The dimensions of cages given in this handbook represent the best attainable under existing conditions, for the size of the cage depends ultimately on the size of the animal house, but they do not compare too favourably with those of the suburban backyard hutch. It is suggested that: (i) in existing animal rooms the interior should be planned so as to allocate as much space as possible to each animal; (ii) favourable consideration should be given to the possibility of enlarging accommodation by means of annexes; (iii) in plans for new laboratories a really generous allocation of space should be given to the animal house; (iv) living conditions should approximate as closely as possible to the animal's natural environment; and (v) the practice of providing a grass run should be more extensively adopted.

Psychological considerations. There appears to be room for a good deal of research into the psychological conditions that make a happy and contented stock. Captive animals may suffer acutely from boredom, and they certainly need exercise, companionship and opportunity for play. Most rodents appear to be agoraphobic and appreciate a nestbox or hut into which they can retreat, and they like to store food. How far such wishes can be gratified depends, no doubt, on experimental requirements such as the prevention of coprophagy in nutrition experiments, but there is room for ingenuity and research in the matter.

Concentration. Overhead requirements like technical equipment and the management of the animal house are easier to satisfy in a large institution than in a small one. Moreover among two thousand licensees there must necessarily be variation in standards, and in a large laboratory any worker whose mind is not sufficiently alive to humane requirements will be brought under the influence of his colleagues. Thus there are great advantages in concentrating research into large departments and institutions.

Home Office staff. In 1943 there were nearly 300,000 experiments per Home Office Inspector, as compared with 19,000 in 1905; there were 577 licensees per Inspector, as against 190 in 1905; and there were 114 registered places per Inspector, as against 38 in 1905; yet on the basis of the 1905 figures the Ram Commission recommended that the number of Inspectors should be doubled.

It would not be feasible to create a huge force of Inspectors for policing the laboratories, and it would not be desirable to relieve research workers of their personal responsibility for ensuring the observance of the pain conditions, but the Inspectors have important duties, especially as regards the allowance of certificates and granting of licences, whereby they set the standards that govern laboratory practice. It seems incredible that any human being, however gifted, could carry efficiently the load imposed by the present size of the staff.

The scientific veterinarian is still regarded as something of a novelty, and old-fashioned medical men find it difficult to believe that he knows as much about animals as they do themselves. Nevertheless there are solid reasons for asserting that veterinarians if included in the inspectorate would be helpful to their medical colleagues, and could give advice to laboratories on animal husbandry. A medical degree is by no means indispensable.

The pain conditions. These relate, as the Ram Commission remarked, to "the difficult region of relative ethics in which standards of what is or is not justifiable vary with different climes, epochs, and temperaments, and in the same persons at different periods of their lives." Although in industry and other non-scientific fields of human activity old injustices die hard and practice lags behind conscience, yet on the whole the under-dog, human or animal, is more generously regarded to-day than he was in 1912, when the present pain conditions were agreed upon. Even then three out of eight signatories of the Ram report recommended that "on the supervention of real or obvious suffering the animal should be forthwith painlessly killed," and long before that T. H. Huxley wrote (*Collected Essays*, vol. 3, p. 310): "I should object to any experimentation which can justly be called painful." There is to-day a strong case for adjusting the pain conditions in a sense favourable to the animals concerned.

In any event the standards prevailing in laboratories are likely to be influenced by those which guide the Home Secretary in allowing or disallowing particular types of experiment. Has he shown enough favour to animals, and is his standard progressive, stationary or retrogressive? Such questions which involve the discussion of particular instances, cannot conveniently be argued in public, but it may reasonably be urged: (1) that the inspectorate and the scientific Advisory Committee should establish a routine for bringing precedents under periodical review, and (2) that they should widen their field of consideration by systematically consulting veterinarians, zoologists and the less emotional sort of animal-protectionists.

Disposal of unwanted invertebrates. Dr. John R. Baker writes: "It repeatedly happens in zoological laboratories that the extra specimens of invertebrates not killed for dissection by the class are just left lying about to die of desiccation, starvation, or the accumulation of products of metabolism. Each class-room might contain a special lethal chamber in which unwanted specimens could be painlessly destroyed."

Use of wild species. Some wild species, such as the wild Norwegian rat, do not take kindly to captivity. Information regarding them has for good reasons been included in this handbook, but without prejudice to the principle that wherever possible laboratory animals ought to be thoroughly tame. When members of wild species are indispensable it is more humane as well as technically sounder to breed them than to capture them.

CHAPTER 2

THE ANIMAL LABORATORY

Compiled from various sources

1. Introduction. 2. The construction of an animal laboratory. 3. Food preparation; washing-up and sterilizing equipment. 4. Cages; cage-equipment; watering; bedding. 5. The supply and identification of laboratory animals. 6. Assistants. 7. The infected-animal room. 8. Some notes on technique and further sources of information (experimental technique; anæsthesia; the sharpening of hypodermic needles). 9. Units of length, weight, volume and temperature.

1. Introduction

The present chapter concerns the construction and fittings of animal ouses suitable for the smaller laboratory mammal, together with some notes n the supply of animals, attendants and technique. Special features of ccommodation and management are dealt with in Chapters 4 to 22, while ests of the animal house and their control form the subject of a separate hapter (Chapter 3).

The design and management of animal laboratories vary enormously, and n some instances the standards are far from satisfactory. It seems futile to xpect reliable results from the use of animals of mixed or unknown origin, mproperly identified within the colony, housed in inadequate, dirty, parasite-ıfested, unevenly heated, badly ventilated, draughty, noisy or otherwise nsuitable surroundings, handled with fear or distaste and fed irregularly on iets containing bulky or rapidly spoiling foods of which the nutritive value as never been ascertained. Too often the room or rooms deemed unsuitable or other purposes are set aside for animal breeding, while the routine feeding nd cleaning are entrusted to very junior members of the lay staff and are egarded as something of a fatigue duty, to be hurried through in order that return may be made to more interesting work. The application of parallel tandards to the chemical or bacteriological aspects of an investigation would e unthinkable although the technical procedures involved are comparable in kill. By contrast it is noteworthy that in many leading laboratories the areful and considerate management of the animal colonies is regarded as ndispensable to experimental success. If this book does anything towards levating the general standard of laboratory animal husbandry, its production ill have been more than justified. Written descriptions and photographs, owever, are not enough. Those undertaking animal management for the rst time or wishing to improve their methods are strongly advised to visit rst-class laboratories, to many of which reference is made in this book.

2. The Construction of an Animal Laboratory

Published accounts of the construction of animal laboratories are few, but rief descriptions of the animal house at the National Institute for Medical

Research, London, and the rat colony building at the Wistar Institute ar given by Wilson Smith (1931, pp. 241–243 and Farris (1942, pp. 5–6) respec tively. Innes (1946) is firmly of the opinion that both small-animal breedin units and experimental animal houses should be one-storied buildings. It i not possible to enter into architectural details, but the general principles in th following private communication from Wood (1944) are so lucidly put that th relevant paragraphs are quoted verbatim. Certain individual points are, c course, subject to some modification or expansion, as indicated later, particu larly for work involving infected animals.

"It is most important to plan the animal laboratory as a whole an to consider its design from every aspect before any work is commenced Wherever possible, it is far better to construct the laboratory as an entit than to endeavour to adapt an existing building. The initial cost may b higher; but this will be offset by the much reduced running costs resultin from ease of maintenance and (as will be seen below) markedly lower heatin costs. Moreover, where parasites are rigidly excluded, complete cleanlines ensured, temperatures controlled thermostatically, etc.—all of which is s much easier in a building designed *ad hoc* than in an adaptation which i perforce a compromise—the work done will be not only easier, but mor efficient and more accurate, which means both a tangible and an intangibl return on the initial outlay of sufficient magnitude to repay the additiona expenditure many times over. The following notes are based on our experienc when in 1937–8 we designed and planned a small animal laboratory, with maximum capacity of about 800–1,000 rats, for vitamin assays and othe nutritional investigations. It will be seen, however, that much of what follow could be adapted without much difficulty to the construction of an anima house in an existing building or even to the modification of one alread existing.

"It is important that the animal house be constructed of such materia and in such a manner, that crevices which can harbour parasites are avoide and regular sweeping out and washing down are facilitated. In ours, the wall are faced with asbestos-cement sheets having what is known as a 'marbled glaze' surface. This is very hard, smooth, and resistant to abrasion, and quit impervious to water. Moreover, it is reasonably cheap in first cost, an maintenance charges thereafter are nil (ours is now six years old and as good a the day it was put in). The material is supplied in 8 ft. × 4 ft. sheets abou $\frac{1}{4}$ in. thick, which in our case are held in position by stainless steel strips 1 in wide, fastened by stainless steel screws to wood battens behind the sheet and covering the line where two sheets meet. There is thus no visible crevic left between the sheets, and no joining material is used. Similar stainless-ste angle pieces are used at the corners of the room.

"The floor of the house (which is long, and divided into sections by part tions) is concrete with a smooth cement finish, and where it meets the walls th rendering is carried a few inches up the wall, giving a rounded finish with n sharp junction-line. There is a slight fall to one side of the room where trough is cut, carrying all water away under the partitions to a drain at on end.

"The rat-cages are of two sizes only, 9 in. × 12 in. for individuals (o occasionally pairs) and 12 in. × 18 in. for mated pairs, mothers with litters, et They are simply constructed of galvanized-wire mesh resting in a galvanize

ray 4 in. deep, with the addition of a false bottom where necessary. These cages are held on racks made of light angle-iron, spot-welded at the joints, and aluminium-painted, in unit sections which take four 9 in. or three 12 in. cages on each of five shelves. The racks are hung from lengths of 1-in. gas piping which run across the room and are held in bearings, which are actually flanged sockets sawn in two and screwed to the walls. Thus the floor is completely free of any obstruction and can be swept or hosed with ease, and the cage-rack sections are interchangeable and light enough to be unhooked and taken down while the walls are washed down with disinfectant and a scouring powder, which is done two or three times a year. All windows are so constructed that no wood is exposed ; the only materials showing a surface in the laboratory interior are cement, glazed asbestos sheets, glass and metal. The success of this method of construction is shown by the fact that the colony has remained completely free from scabies, lice, or any other parasitic infection since its inception 6 years ago (with one exception—see section 4 below). All the materials used are reasonably cheap and the labour involved in construction is not great. The stainless steel strips were relatively the dearest feature and could no doubt have been replaced by a cheaper metal ; but they are admirably suited to their purpose, should outlast the other components of the laboratory, and certainly enhance its appearance.

" If the animal house is being constructed *ab initio*, it is well worth while to provide heat insulation behind the walls ; the initial cost will be quickly repaid by the saving on fuel costs. In our case, the treatment employed was to line the outer walls with aluminium foil, which can be obtained for the purpose reinforced with asbestos. Half-inch battens are then fastened over this horizontally and vertically so as to divide the walls into 24 in. × 24 in. squares. A second layer of foil is stretched over this and secured with $\frac{1}{2}$-in. battens laid over the previous ones. The final wall surface (in our case the asbestos-cement sheets) is then fastened to these battens. Between the outer walls and the inner surface one thus has two $\frac{1}{2}$-in. spaces of (practically) ' dead air ' and three bright reflecting surfaces. The manufacturers of the foil claim that the insulating power of this combination is at least equal to 4 in. of cork. We have no data on this point, but our heating costs have certainly been remarkably low ; and would undoubtedly have been lower still had it occurred to us (as it should have done) to have double windows—an alteration we shall certainly make after the war.

" The above treatment is all the more necessary and effective if used in conjunction with the particular method of heating we employ. We set out to maintain our laboratory at a thermostatically-controlled temperature of 70° F. in the experimental sections (housing rats receiving special diets) and 75° F. in the breeding sections. The system eventually installed depends on hot-water radiators fed from a large lagged tank outside the laboratory proper and heated by electric immersion heaters. A master thermostat inside the main experimental section operates a valve (of the ' all-or-none ' type) which cuts off or permits circulation of hot water from the tank and simultaneously switches off or on the immersion heaters, so that heat is put into the tank whenever it is withdrawn. Subsidiary simple thermostats on each radiator separately enable other sections of the laboratory to be held at a different temperature from the main sections. There are other ingenious refinements of which space forbids description here. The lagged tank has sufficient reserve

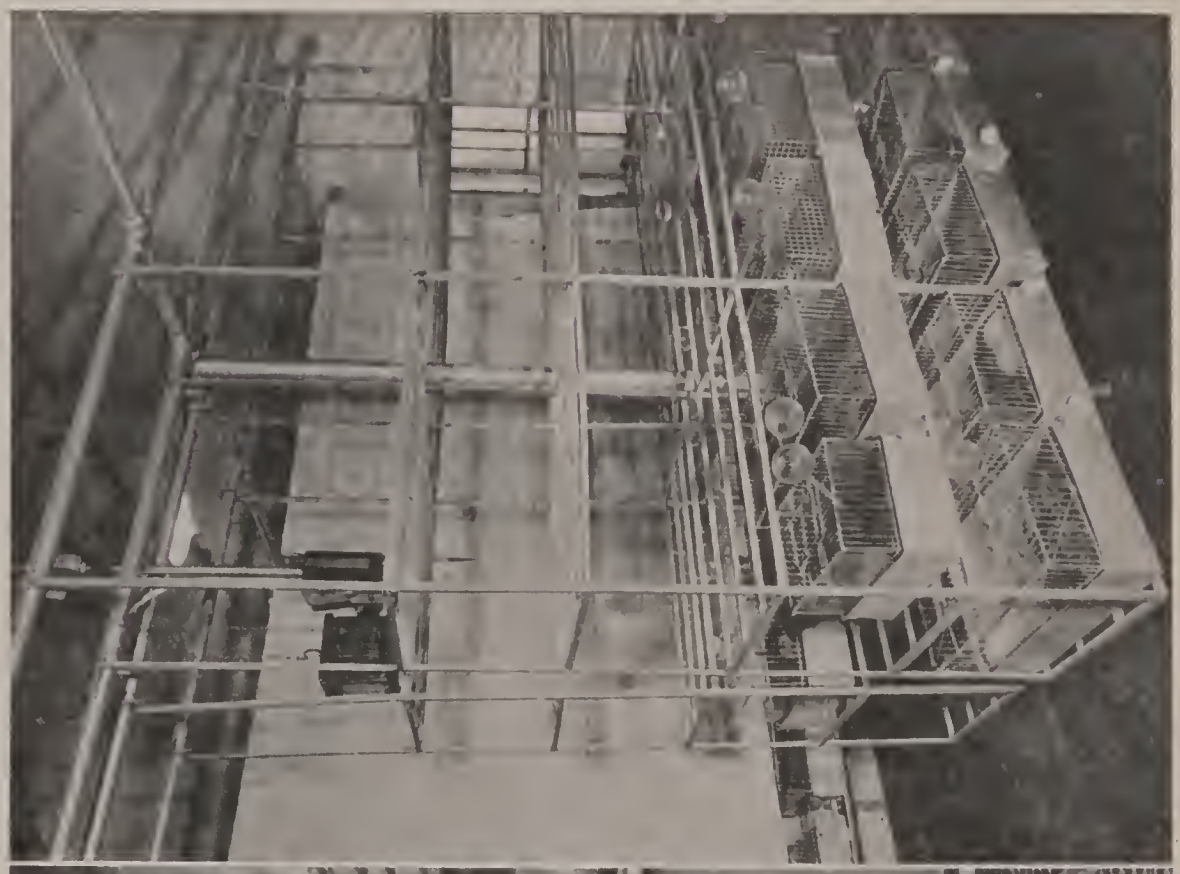


Fig. 2.—Cage-Rack Design at the Virol Laboratory.

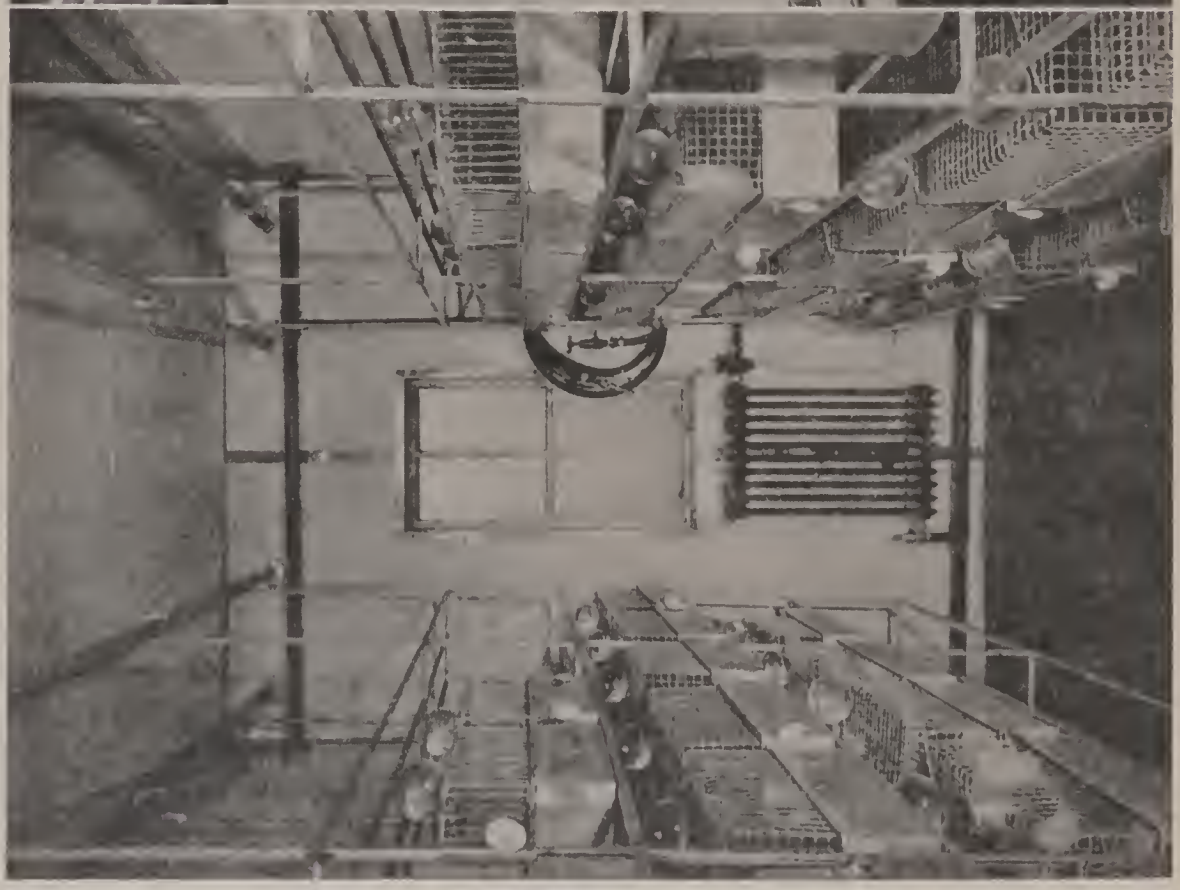


Fig. 1.—Section of the Animal Laboratory of Virol Ltd.

capacity to provide heat for the laboratory for several hours even if the electricity supply is cut off ; which not only acts as a safeguard against failures but also makes it possible to have the supply deliberately switched off by a clock-controlled switch at pre-set ' peak ' periods—and the electricity supply companies will usually provide such ' off-peak ' power at a very cheap rate. The installation, if put in at the time the laboratory is built, is not at all costly, and heating charges from then on are very pleasantly low. Failures due to mechanical or electrical faults are very rare ; failures of supply are, as stated above, without effect unless lasting more than four hours (which has never happened to us yet) ; and the thermostatic control is as near perfect as may be."

A single section of Wood's laboratory is shown in Fig. 1. The main points to note are : (*a*) the hot-water radiator with the thermostatically-controlled valve which maintains an even temperature ; (*b*) the coiled hose on the wall by the window with which the floor can readily be washed down ; (*c*) the freedom of the floor from all obstruction ; (*d*) the construction of the racks on which the cages are hung (this, however, is better seen in Fig. 2) ; (*e*) the half-flanges screwed to the walls which support the pipes from which the racks are hung ; (*f*) the construction of the wall and window. Note the metal sheathing around the window which prevents the exposure of any wood.

An *air-conditioning plant* is used in the animal houses of some up-to-date laboratories in the U.S.A. (Herrlein, 1940, pp. 5–6 ; Farris, 1942, p. 5) and Great Britain (Jones, 1944). Herrlein states that ideal results have been obtained by use of a circuit in which air enters at floor level and is discharged at ceiling height. With such a circuit he has been able to ensure a constant supply of fresh air which can be maintained at a temperature of approximately 70° F., and with a humidity range of 40 to 70 per cent. throughout the year. The disadvantage of the circuit in which air enters near the ceiling and leaves near floor level is that it exposes the animals, whose cages are usually open at the top and/or sides, to down draughts. Jones, who advocates a similar circuit to that described by Herrlein, employs a rate of circulation which is sufficient to effect about six complete changes per hour, and finds that this keeps the atmosphere of the animal house quite fresh and comparatively free from smell. Wood (1944) uses forced ventilation, with a suction fan operating on exhaust ducting near the ceiling, the inlets being at floor level. The experimental room at 70° F. takes in air from the breeding-room at 65° F., which itself takes in air from the room where weighing, etc., is done ; and the latter takes in air from outside the laboratory via openings *behind the radiators*. The air is thus most efficiently pre-heated. In all rooms there are windows which can be opened, but these are opened only on hot summer days.

When a building or room is being converted for animal use the simplest effective method of providing a thermostatically controlled heating system is probably by the introduction of *electrical tubular heaters*. These are inexpensive and give very satisfactory results. For emergency or temporary use small electric or other stoves may be used, providing the ventilation and temperature are judiciously controlled and draughts are avoided ; a well-constructed room is absolutely essential for such to be achieved.

A *mobile heating unit*, which can be assembled easily in the laboratory workshop, has been used at the Cooper Field Research Station, Little Gaddesden, Herts (Pollard, 1946).

The apparatus consists essentially of a cylinder of compressed asbestos

(1 ft. diameter by 1 ft. length), carrying an internal lattice arrangement of tw or three spiral heating elements of the type used in the ordinary domest electric fire. Air is circulated through this and around the room by an electr fan placed immediately behind it ; the whole being mounted on a wooden ba about 1 ft. 6 in. wide by 1 ft. long. The thermostatic make-and-break mounted laterally behind the fan to control the heating circuit. The fan kept running continuously.

FIG. 3.—SMALL RABBIT CAGE AND TRUCK DESCRIBED BY HERRLEIN.

The truck or trolley is of angle-iron construction, all welded and hot-dip galvanized af fabrication. Note rubber-tread ball-bearing swivel casters. The pans and slide flo are both removable on independent slides.

This method will not maintain a high temperature in very cold weather, b is useful to supplement and control the temperature in rooms which alrea have some uncontrolled source of heat. Its chief virtues are its mobility a the fact that, by circulating hot air, it quickly establishes a uniform temper ture throughout the room.

As is emphasized in later chapters, it is usually desirable to mainta breeding and experimental animals in separate rooms, and also to keep o species only in any one room. Design should therefore make for the short

ossible corridor space through which animals have to be transported from one bom to another. Moreover such corridors should so far as is practicable be haintained within the same temperature range as that of the animal rooms hemselves. The corridors as well as other parts of the animal house should if ossible be made *fly-proof*.

Artificial light is essential. Wells (1940) has developed an inexpensive type f lamp which lets down a curtain of ultra-violet rays in the room and is intended o destroy bacteria. It may be substituted for an ordinary electric light and arris agrees that it may prove ery useful in preventing air-orne infections. In the animal ouse described by Wilson Smith ne wires are of the marine type nd all switches are on the corri-or walls and are water-tight and amp-proof.

Fixed *cage racks* with upright anchions fixed into the room-oor have certain disadvantages. hese are largely overcome in the esign recommended by Wood, oove (see Fig. 2). The best idea, owever, would seem to be that cage-trolleys (see Fig. 3). hese have the advantages that ey may be used for conveying imals from room to room with e minimum of disturbance, and n be removed altogether for parate cleaning or sterilization, aving the room itself virtually eared for cleaning purposes. At e South African Institute for edical Research, Johannesburg, d at the Onderstepoort Veteri-ry Research Laboratories, the dinary type of cage and cage-rack has been replaced in some instances wire-floored cages suspended 24 in. above the floor from horizontal bars Iason, 1944). The fæces and urine of the animals concerned (rabbits and inea-pigs) drop together with waste food on to the floor, which is covered with layer of wood shavings or sawdust. It is claimed that much labour is saved, e cages are always clean, and there is practically no odour in the room. In rtain circumstances this system may be most useful, but it would seem unwise attempt to apply it to the murine rodents which demand a constant temperature environment of about 65° to 70° F. The automatic removal of soil d waste matter may be effected in their case by the Wistar Institute method erred to in Chapter 6, pp. 112–113.

[*Photo. W. J. Smith*

FIG. 4.—AUTOMATIC BALANCE USED AT THE INSTITUTE OF ANIMAL PATHOLOGY, UNIVERSITY OF CAMBRIDGE.

A movable *table*, with a top of slate or damp-resistant metal, is suitable the examination and dosing of animals, and for note recording preferable to

An *automatic balance*, for weighing the animals (see Fig. 4)

other types as it makes for speed and great accuracy. Such a balance is be kept on a slate ledge, as indicated in the illustration.

A useful adjunct to any animal room used for murine rodents is the " simp floor net " designed by Keeler (1941) to capture the escaped laboratory roder The value of this device, which consists of a specially made, blacked net standi flush against the floor and wall, is stressed by Meyer & Meyer (1944) wh provide an illustration. The escaped animal, on seeing the dark openin enters and is caught.

3. Food Preparation; Washing-up and Sterilizing Equipment

A separate *food-kitchen*, which may be kept at a temperature more suited the staff than is that of the animal rooms, should be equipped with *hot and co water*, adequate *sink accommodation*, easily-cleaned *cupboards* and a cool annex in which foods may be stored in *bins or other metal containers*, preferably su pended on metal racks similar to those used for cages. The tables should ha tops of slate or of damp-resistant metal. Balances of the automatic ty (similar to that illustrated in Fig. 4 but with scoop-shaped pans) are mo suitable for weighing food. The sinks and draining-boards may well constructed, as in many modern domestic kitchens, of damp-resistant metal.

The cleaning of cages should also be carried out in a separate room annexe and should be carefully supervised. Adequate hot water, soa disinfectant and brushes are necessary. A stiff-bristled pointed brush is advantage in scrubbing the corners of cages. Cages and cage fittings and utensils must be cleansed rigorously. If routine sterilization in an autocla is not practicable, infected cages should be soaked in disinfectant after thorou cleaning. The length of immersion in the disinfectant will depend upon nature and strength. Lysol in concentration up to 5 per cent. is one of t many disinfectant solutions in use.

For work with infectious agents a cage-autoclave is essential, and there little reason why the procedure should not be extended to cages used f nutritional and other work in which infections can ruin an experiment, wh the risk of ectoparasites is greatly reduced when cages are sterilized routinel Sterilization is not, however, to be regarded as a substitute for cleaning, b only as a complementary procedure. Really good cleaning may make t omission of sterilization permissible in non-infective work but the reverse c never be true.

4. Cages; Cage-Equipment; Watering; Bedding

In the absence of some good reason to the contrary, all-metal *cages* a desirable. The effective cleaning and sterilization of non-metal cages—oth than those made of glass, which are preferred for some purposes—prese difficulties which are not likely to be overcome with ease at the routine lev Adequate reasons for the provision of easily cleaned and sterilized cages a given in Chapters 3 and 6.

Perhaps the simplest type of cage suitable for routine use is that describ by Wilson Smith (1931, pp. 244–5; Figs. 72 and 74) for rats and rabbits. this type the roof serves also as a lid and the cage proper, which is made iron-wire, rests in a metal tray in which peat or bedding material is place The loose grid floor, illustrated in the case of the rabbit cage, is desirable

ost work as it facilitates cleaning and leads to the elimination of intestinal arasites. (In nutritional experiments it also reduces coprophagy.) Other mple types of cages which have proved of value for various species are described nd illustrated in the sections on *Accommodation* in Chapters 4 to 17. There is ill need for improvement in cage design and construction, many present-day ges being not only difficult to keep ean but also too small to permit adequate exercise.* McIntosh 942) has published detailed original esigns of both stock and breeding ges for rabbits, guinea-pigs and rrets. McIntosh states that among e most desirable features of animal ges is that they be assembled in its for the conservation of space, ford the animals contained a maxi-um of comfort, while providing for se of observation and the main-nance of sanitary conditions with a inimum of effort. He has given rticular attention to the improve-ent of the design of cage door, placing the conventional small-nged or slide door common to inea-pig or rabbit cages with one at extends the full width of the ge and remains in either the open the closed position without the l of latches or catches. It is ened by tilting upwards and back-rds into the top of the compart-ent and thus is out of the way en the animals are being fed or ndled and permits a full and un-structured view of the cage interior. her features of the cages are strated in Figs. 5, 6, 7, 8, 9, 10, and 12. The use of electric weld ric in conjunction with a partial id wall is stated to provide for ple ventilation without cross aughts, to admit of an abundance light to the cage interior, and to offer little obstruction to the visual exami-tion of the animals.

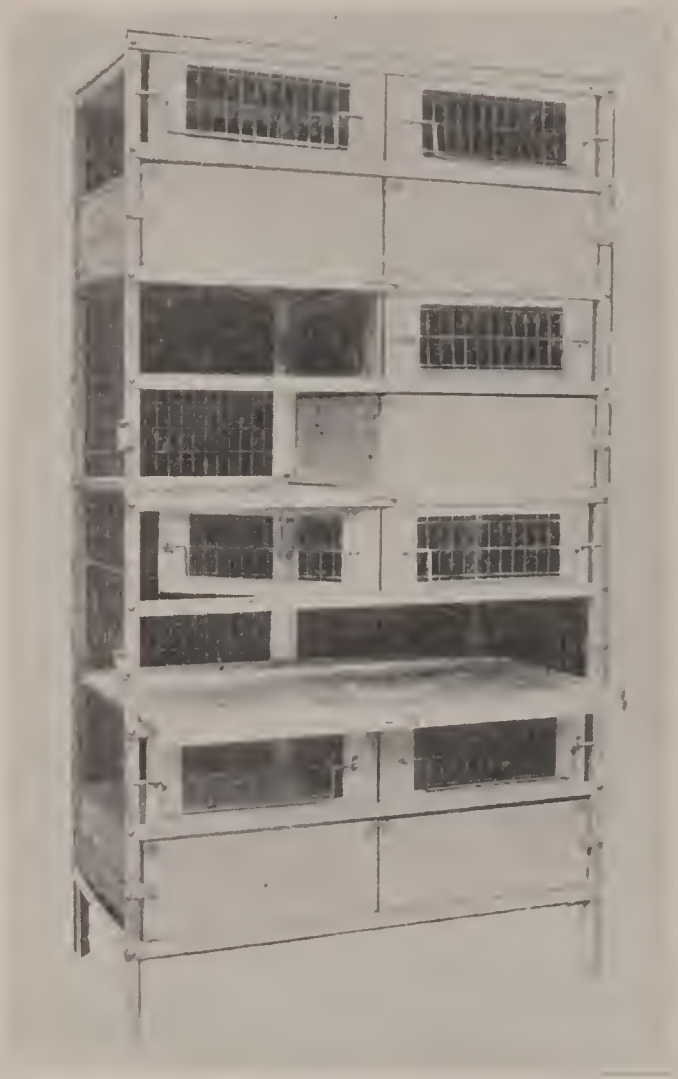


FIG. 5.—FERRET STOCK CAGE DESIGNED BY McINTOSH.

For details see Figs. 6 and 12.

Figs 5–12 are reproduced by the courtesy of Dr. Mitchell, Dominion Pathologist, Dr. C. W. McIntosh, and Officials of the Dominion Department of Agriculture, Quebec.

The murine rodents prefer to have some form of *shelter*. Greenhall (1944)

* Plans have recently been seen of the prototype cage designed by the Northern Dairy gineers, Ltd., Clough Road, Hull, in collaboration with Professor Paul G. 'Espinasse, iversity College, Hull. The features of this all-metal cage, which is constructed from terial similar to that used in the manufacture of racks for carrying milk bottles, are at durability and considerable adaptability. Further details may be obtained from se named.

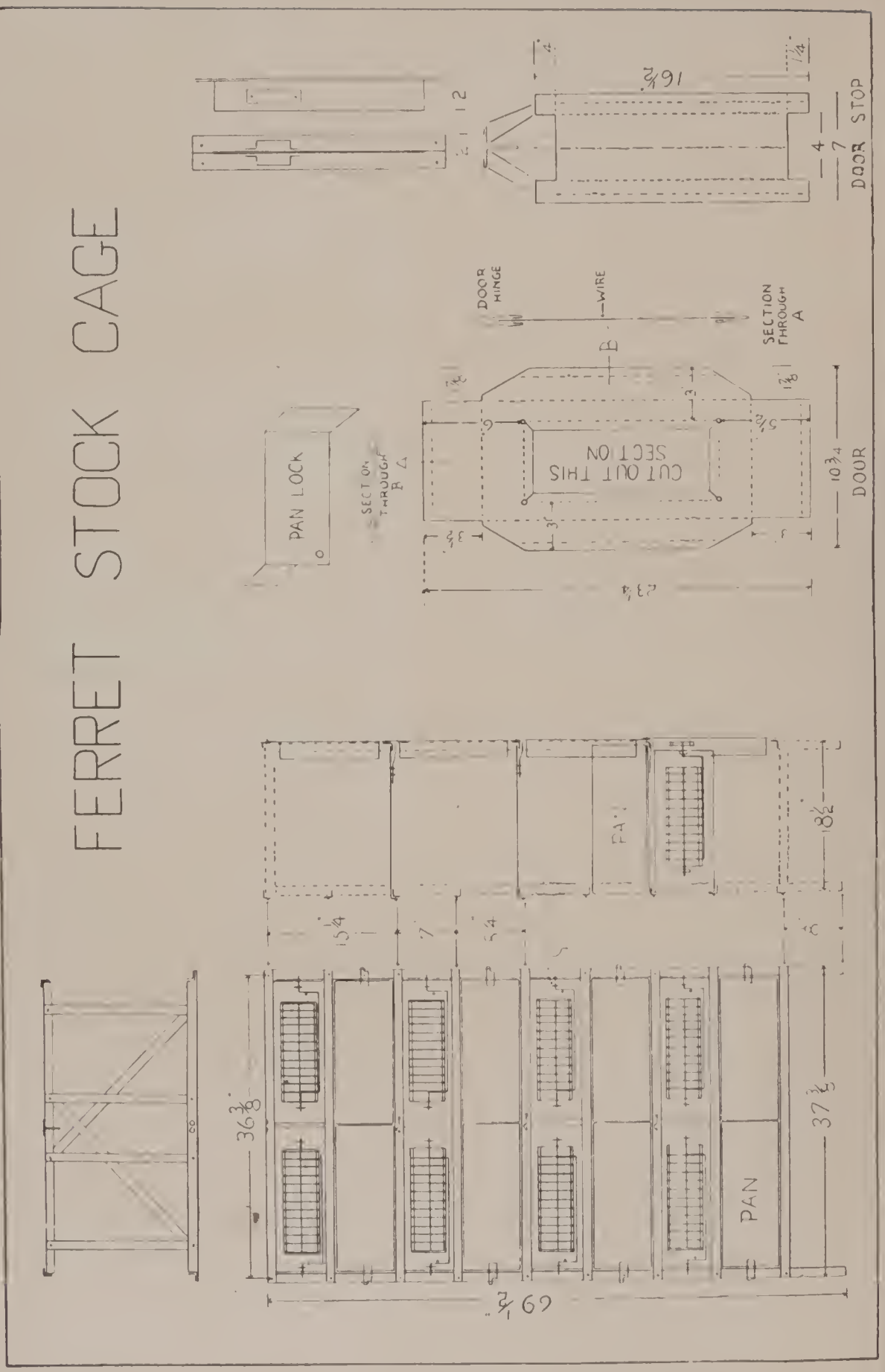


FIG. 6.—PLAN OF THE FERRET STOCK CAGE ILLUSTRATED IN FIG. 5.

FIG. 7.—FERRET BREEDING CAGE DESIGNED BY MCINTOSH.

For structural details see Fig. 12.

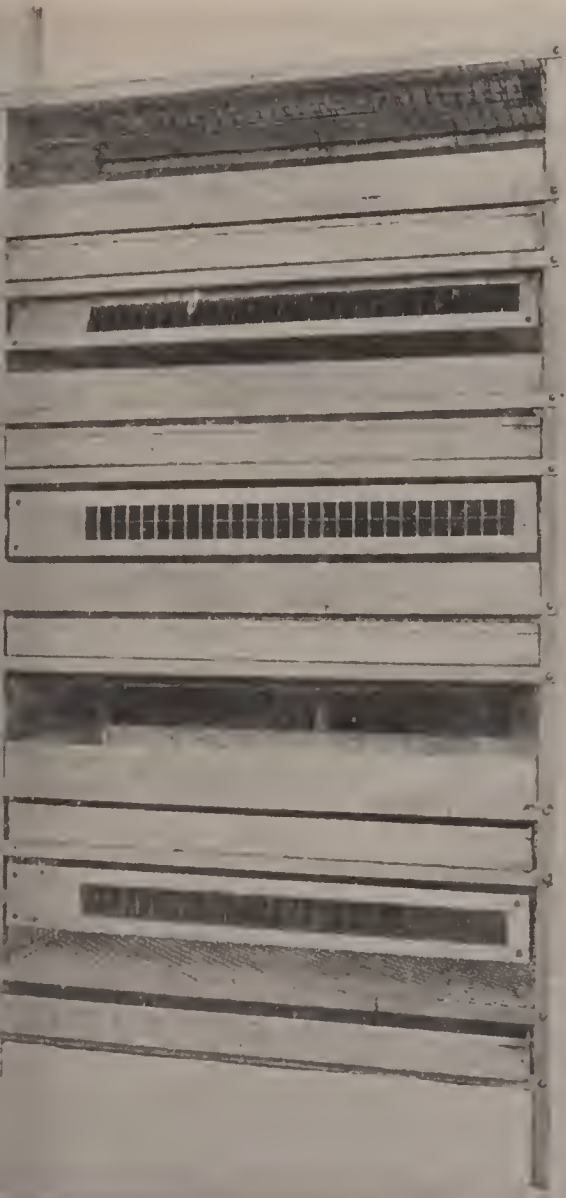


FIG. 8.—GUINEA-PIG STOCK CAGE DESIGNED BY MCINTOSH.

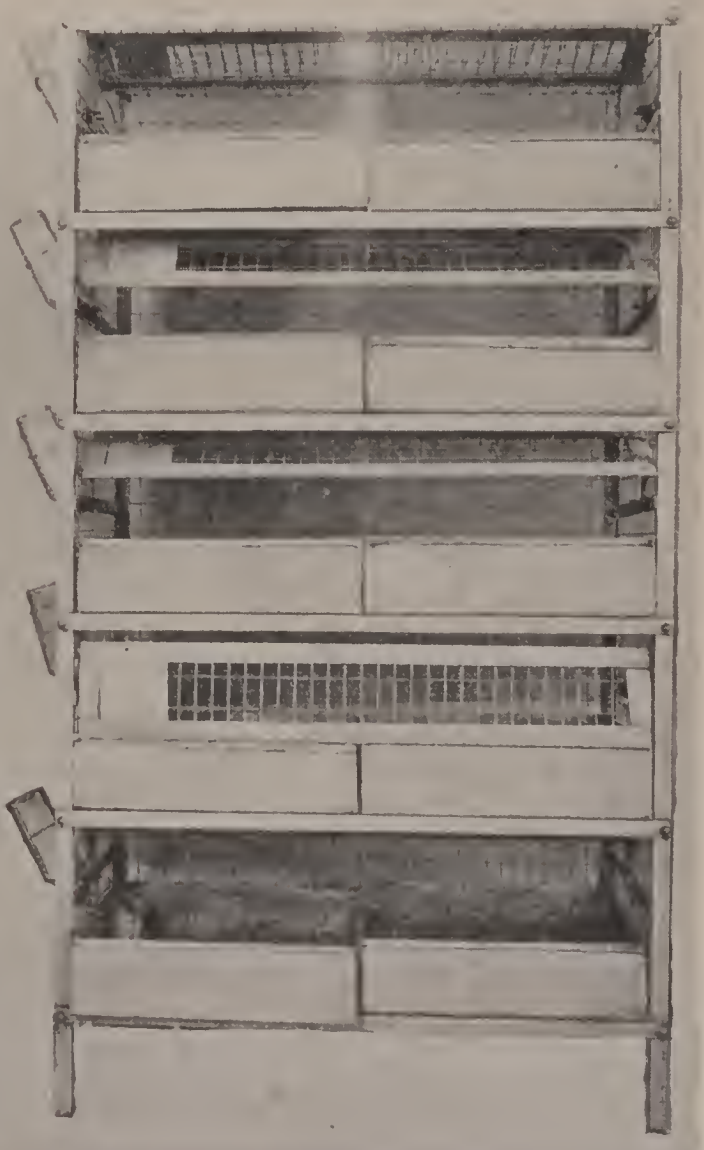
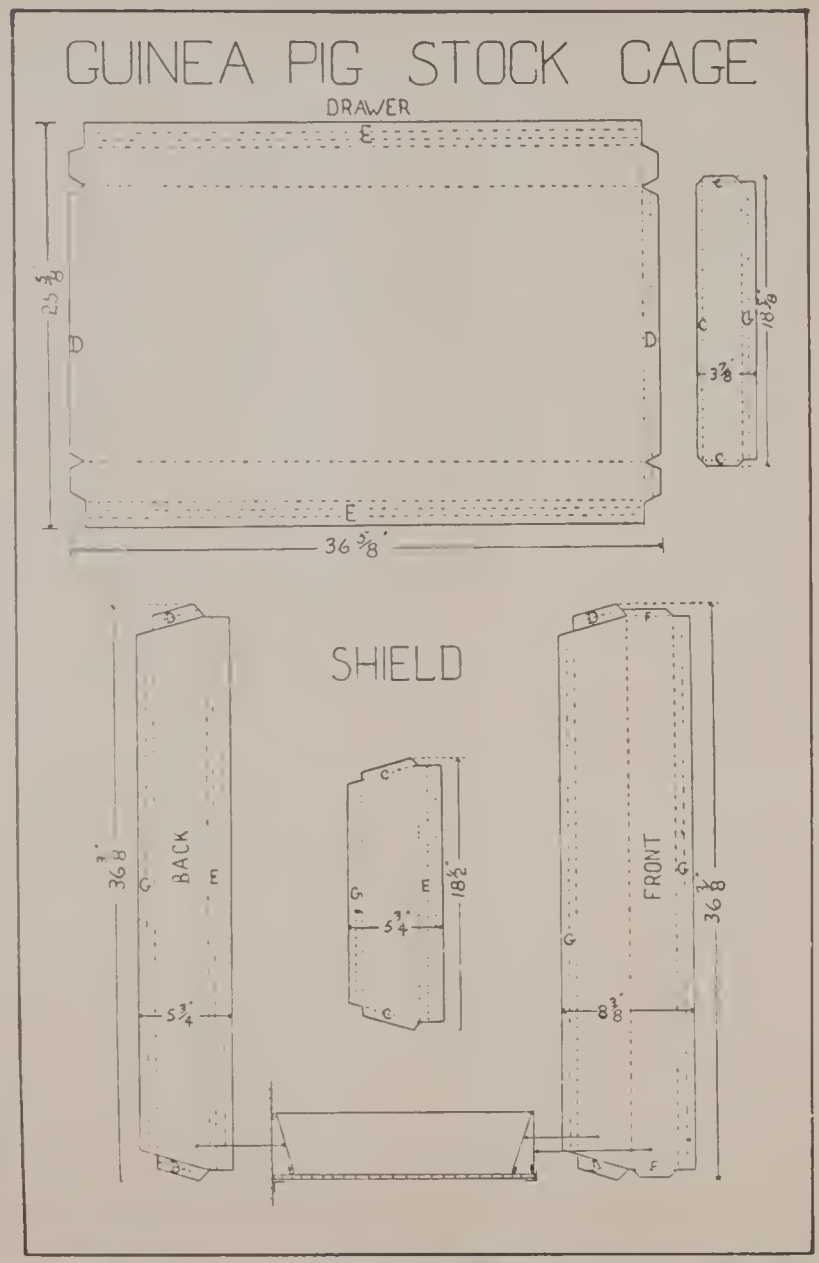


FIG. 9.—GUINEA-PIG BREEDING CAGE DESIGNED BY MCINTOSH

For structural details see Figs. 10 and 11.



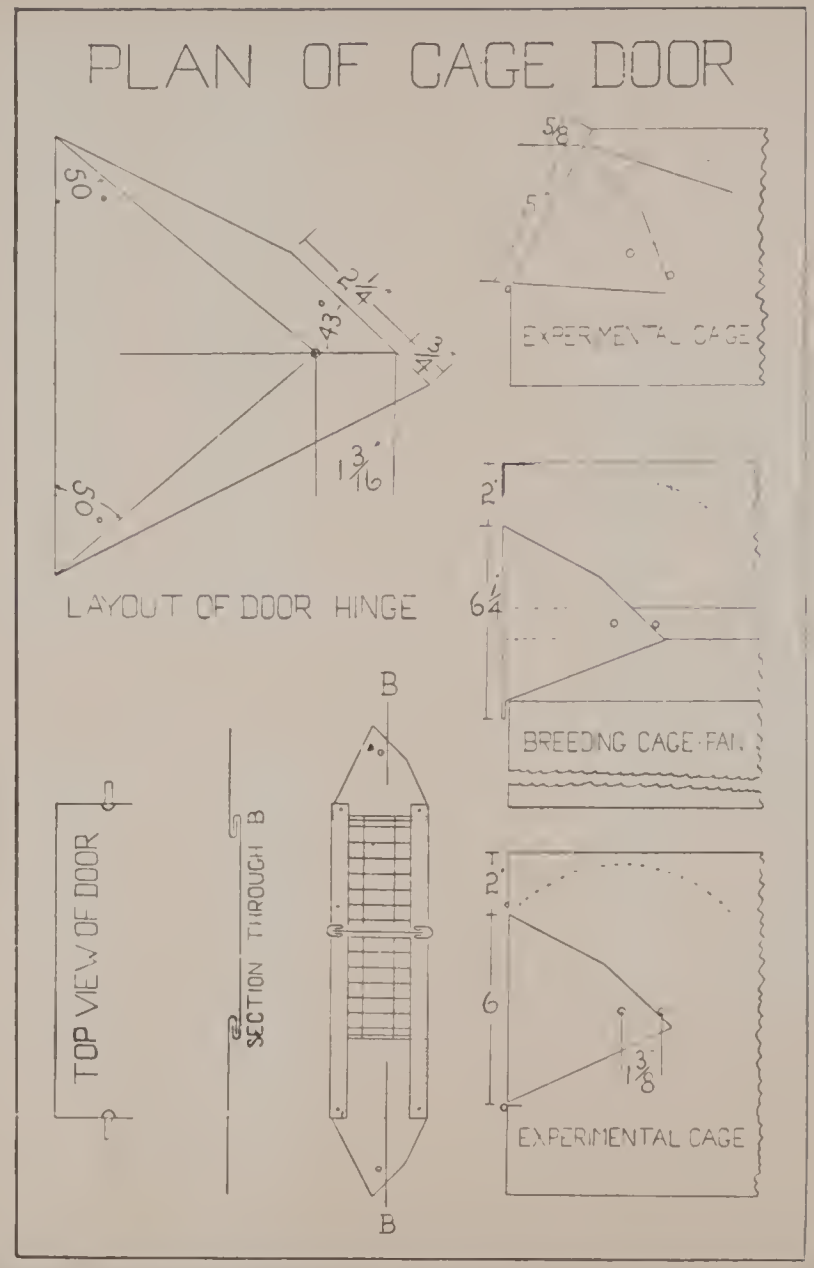


FIG. 10.—DETAILS OF GUINEA-PIG STOCK CAGE ILLUSTRATED IN FIG. 8.

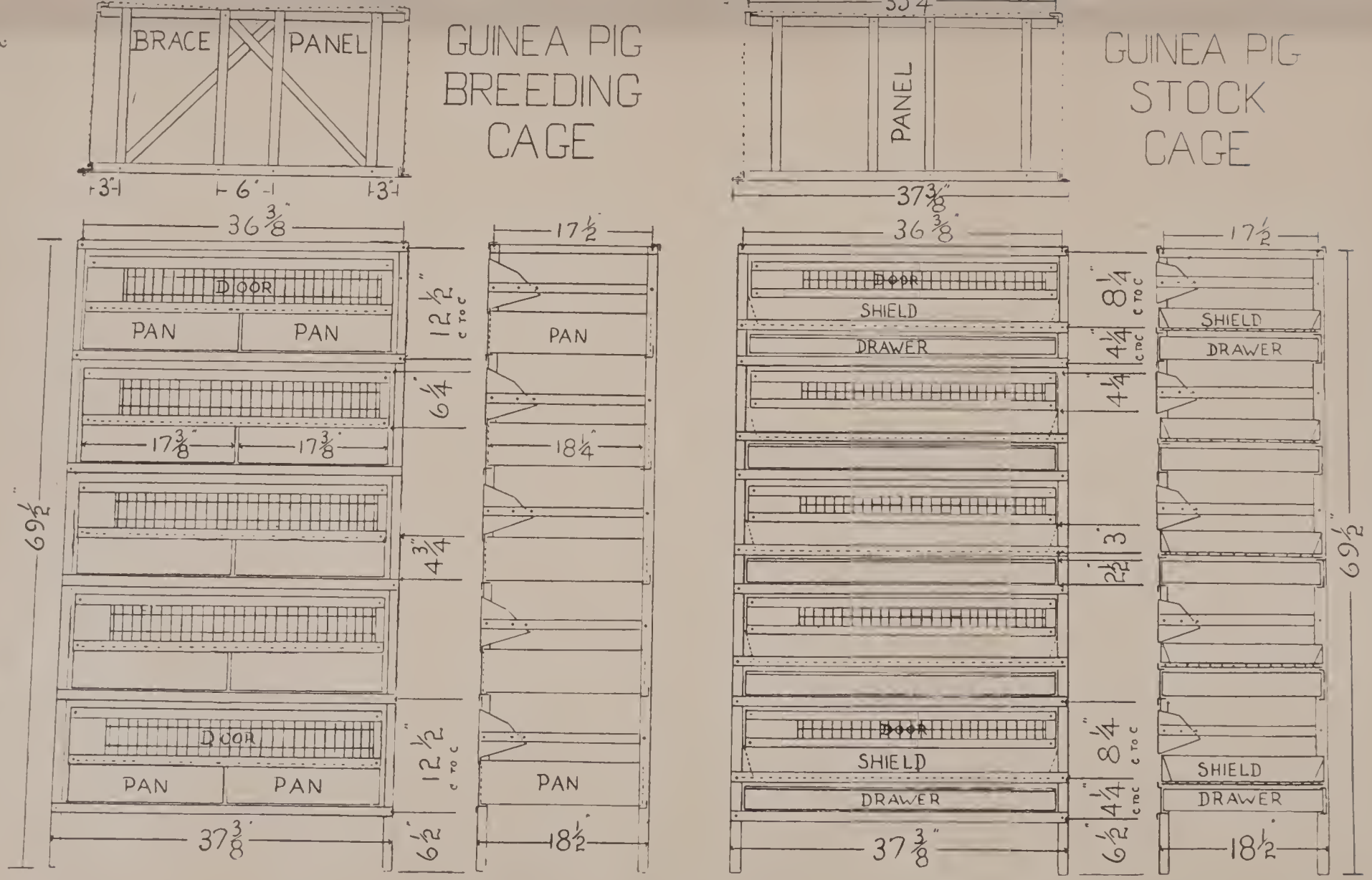


FIG. 11.—STRUCTURAL DETAILS OF CAGES ILLUSTRATED IN FIGS. 8 AND 9.

Guinea-pig cage

Ferret cage

Ferret breeding cage

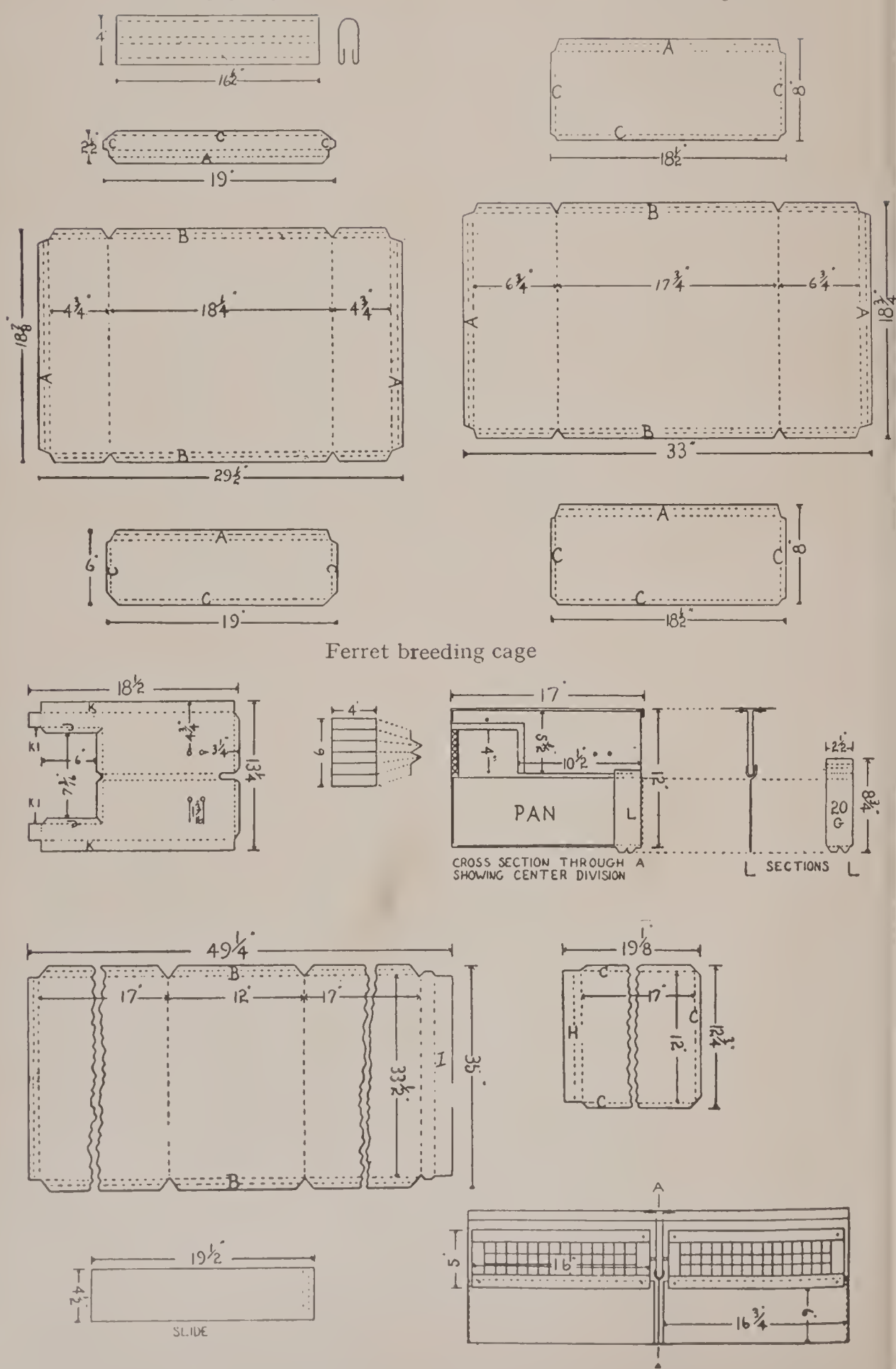


FIG. 12.—STRUCTURAL DETAILS OF CAGES ILLUSTRATED IN FIGS. 5, 7, 8 AND 9.

tates that unless some of the small rodents brought to the Portland Zoological Park are provided with shelters, they die of nervous prostration. Even the horoughly domesticated rat likes to have some secluded retreat (see Chapter 6, . 117, and Fig. 29). Permission to hoard is also desired by many rodents. These psychological considerations are important not only for captive wild nimals (see Penrose's introduction to the textbook of Fox, 1923; Walker, 942), but also for species which have been bred in captivity for many genera- ons. Animals which are bored, repressed or otherwise unhappy may fail to reed and cannot be considered as adequate experimental material (Edwards, 945).

Two forms of *exercising-disc* are depicted in Fig. 13. They represent two ypes selected by Walker (1945), who has experimented with this subject for ome time. The inclined disc can be made of ply-wood, a hard pressed fibrous naterial such as Masonite, or composition plastic. The bases of the disc-heels should be wide enough to project beyond the edges of the wheel; therwise the wheel may work gradually against the edge of the cages and free novement be retarded. The Farris wheel of cylindrical type, also shown in ig. 13, should have a diameter equal to at least three times the head and body ngth of the animal that is to use it. The clearance of this wheel from the oor of the cage should be about the diameter of the body of the animal to revent young animals from being caught under it. Relatively small surfaces the bases rest on the floor, thereby facilitating cleaning. In the large size isc the corners of the box are turned downward slightly so that the principal earing is at these four points. The principal framework of both types is made No. 7 electrical galvanizing to withstand eight 1-minute immersions in the opper sulphate used in the Preece test. The disadvantages of exercising heels are that:

(i) they may hinder effective cleaning of the cage;
(ii) in nutritional experiments, in which weight changes are measured, unequal exercise would invalidate the results. Psychologically, however, they are of undoubted value, especially when cage room is none too plentiful. As mentioned in Chapter 6, p. 118, modifications of exercising wheels suitable for activity records, and exercising cages proper, are described and illustrated by Farris (1942, pp. 9–10).

An adequate *water-supply* is essential for all classes of experimental animal. or the murine rodents, and even for rabbits and guinea-pigs, fountains or ulbs are almost always preferable to pots or bowls. The open vessel is easily oset and its contents spilt or contaminated with faeces. Two types of water- n be seen in Figs. 14A and 14B, the bulb is complete except where the em enters it, and some difficulty is experienced in filling. Fig. 14B is a notograph of a device designed by Wood (1944) for the filling of water-bulbs. ne horizontal tube running across the top is pierced in four places and has a ort length of $\frac{1}{8}$-in. brass tubing attached to it. This is attached by a few ches of rubber pressure-tubing to a further length of $\frac{1}{8}$-in. brass tubing long ough to reach well into the bulb of the water-bottle when the latter is on the elf, as shown. The brass tubing is slipped through the stem of a small verted glass funnel which is then secured at the top with a piece of adhesive pe. This acts as a splash-hood when the device is in use. In LBS. the

girl filling the water-bottle turns on the water supply and slips the bottles o
the brass tubes, beginning at the left-hand side, and by the time the fou
bottle has been put on the first is full, so that the process can be made pr

FIG. 13.—WHEELS AND DISCS FOR EXERCISE.

tically continuous. A second type of bulb, which has every advantage,
that illustrated in Fig. 15. This type may be flushed out daily from a runn
tap b e re-filling. Mechanical watering devices are used in some labo
torie at the Wistar Institute is illustrated by Farris (1942). A sim

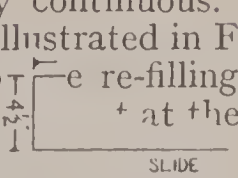


FIG. 14A.—RABBIT DRINKING FROM WATER-BULB. CHESTER BEATTY RESEARCH INSTITUTE.

FIG. 14B.—APPARATUS DESIGNED BY WOOD FOR FILLING WATER-BULBS. For details see text.

design is that of Mason (1944). With any common-supply system great ca must be taken to avoid transference of infection from cage to cage.

The subject of food receptacles for routine use is dealt with in Chapter p. 116, and elsewhere under individual species. (See also section 7 below.)

Cages must be adequately labelled. It is desirable that each cage, as ea animal in individual experiments, should bear its own number, but in additi a detachable metal label-holder should be provided. The simplest hol consists of a metal sheet, with edges turned over to enable a card, beari details of the animal, experiment and licence-holder, to be slipped in, and me clips to secure it to the cage. Neither holder nor label should fall easily fr the cage. The tying-on of labels with string is not always satisfactory, especia with species which gnaw, while chalk or pencil marks are too eas erased. Identification labels must be changed with great care wh experimental animals are changed from one cage to another, as th may be as often as twice weekly in summer when flies have gain access to the animal room. (The label should be signed by t licence-holder.)

FIG. 15. —WATER-BULB SUITABLE FOR USE WITH LABORATORY RODENTS.

As Herrlein (1940, pp. 26–27) states, clean (uncontaminat and proper *bedding* is as important as proper housing, caging a diets, and should be secured from reliable sources where measu are taken against exposure to wild mice, wild rats and house ca He advocates coarse white pinewood-shavings, baled at the m for rats and mice, and agricultural peat moss—also baled—f rabbits and guinea-pigs. One should take precautionary measu against contamination as with food. Herrlein does not mention t need for routine sterilization but even with all possible precautio as to origin and subsequent treatment all bedding should, as at t Wistar Institute and at many bacteriological laboratories, sterilized before being taken into the colony (see Chapter 6, p. 11 Flies, lice and many other parasites may be introduced by co taminated bedding.

5. The Supply and Identification of Laboratory Animals

The supply of animals for laboratory use has been a matter concern for many years. Certain laboratories have bred their ov stocks of some or all of the species they employ, while in a few cas such laboratories have produced sufficient quantities of uniform and healt animals to supply other workers with foundation or experimental stock. T proportion of laboratory animals bred in this way is, however, far too sma The purchase of stock is usually a somewhat indiscriminate affair, involvi transactions with private breeders of very varying capability and resourc quantitative and qualitative fluctuations in the supply, enhanced risks introducing disease, and almost complete ignorance of genetical constitutio The result is great lack of uniformity in many stocks and consequent inabili to reproduce reliable results. From the humanitarian point of view also, su practices are open to grave objections. The smaller species may have be bred in far from ideal surroundings, while much public suspicion would allayed if the origins of all cats and dogs employed in the laboratory wo accurately defined. There can be little doubt, for example, that the do

ecially bred for the canine distemper research at the Farm Laboratories of e National Institute for Medical Research were temperamentally better ited to experimental work than dogs acquired at random by certain workers. nese remarks must not be taken to imply that commercially produced labor- ory animals are necessarily inferior to laboratory-bred stock. Indeed some ms, as is evident from the contents of this book, breed animals of extremely gh standard and with scientific control.

In an attempt to clarify some of the problems involved, a *Conference on the pply of Experimental Animals* was set up in 1943. The Conference was sup- rted by the Anatomical, Biochemical, Genetical, Medical Research, Nutrition, thological, Pharmacological, and Physiological Societies, the Association of inical Pathologists, Association of Scientific Workers, National Veterinary edical Association, Royal College of Veterinary Surgeons, and Royal Society Medicine (Sections of Therapeutics and of Comparative Medicine). The Hon. cretary of the Standing Committee was Mr. A. L. Bacharach, M.A., F.I.C., , Willow Road, N.W.3. (The recommendations of the Conference were sent nfidentially to the Secretaries of all supporting Societies at the end of 1945.)

Within a given stock the individual identification of animals is usually of eat importance and is essential where breeding records are kept. During tual experimentation the marking of an animal as well as of its cage is a feguard which it is usually wise to adopt. The different systems of marking clude recording of coat-pattern (Chapter 5, p. 102), tattooing, daubing with fferent coloured dyes, ringing the legs, tagging the ears, or making minor utilations such as nicking the ear, etc. Leg-rings are suitable for birds but t for mammals, while dyes are usually unsatisfactory. Ear tags are usually erior to carefully tattooed numbers, but with very small mammals neither vice is practicable. Ear-snips or punches are widely applicable. Keeler 940) has devised an ear-punch which is stated to be capable of recording to 10,000 by suitable combinations. Wood (1944) uses a number code (for ntifying rats) in which the offspring of a given doe are numbered successively 02, 03, etc., each new litter beginning where the previous one left off. The lividual's number is then combined with its mother's, maternal grandmother's, ., numbers for as many generations as are needed for the purpose in hand, gether with a generation number, reading from right to left. Thus, rat mber 19.06.14.02 is a rat of the 19th generation, the second rat born to a ther who was the 14th rat of her mother who was the 6th rat of her mother. e pedigree card is so kept as to facilitate looking up the particulars of any rat ntified this way. In addition, to distinguish between animals at sight, an -marking scheme is used in which the rats are marked (while under light oroform anæsthesia) with a special punch which removes either a small cle or an oblong from the ear tissue. (N.B.—Remarks on cutting ear sue in Chapter 6, p. 128.) If not more than two marks are used in each ear different markings are possible ; if three marks are used each ear provides different markings. The ear-punch is of a design made specially for purpose (Information on application to Dr. E. C. Wood), and it has ved most convenient in use. This punch is illustrated in Fig. 16. The lars P1 and P2 are fixed to the arm A by bolts passing through clearance es in A and screwing into tapped holes in the pillars, the heads being counter- nk. A forms one handle and the other is provided by the arm B, which is voted in the pillar P2. P1 provides a bearing for this arm and stops any

possibility of sideplay. In the same way the bar C is pivoted on a pin passi through P1 and is free to move inside P2. The arm A carries at its tip a sm circular anvil, while C similarly carries a hollow nipple N with sharpened edg This nipple screws into a hole in C, and that part of it which is immediate above the cone-shaped cutting portion is provided with " flats," so that t nipple can be very easily screwed in and out with a small spanner. The nippl are interchangeable and designed to give either circular, triangular or oblo holes, which are very readily distinguishable from each other. The dimensio of the various parts are so arranged that when the nipple is in contact with t anvil the sharpened edge of the former is accurately in parallel alignment wi

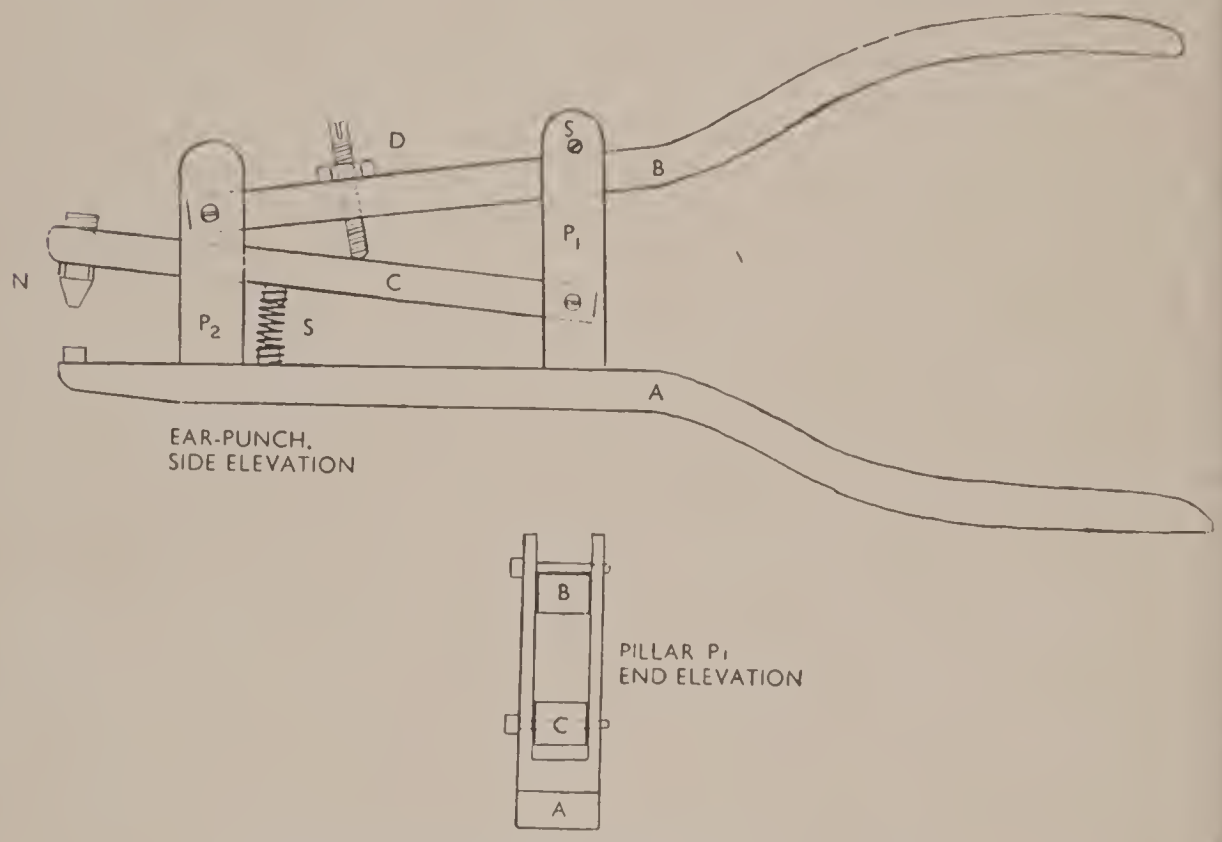


FIG. 16.—EAR-PUNCH DESIGNED BY E. C. WOOD.

the face of the latter ; thus a clean hole is very readily punched in the rat's e without any tearing, and with but light pressure by the operator. S is a spri which opens the gap between the nipple and anvil when the pressure is remove and D is a screw provided with a locknut for initially setting the maximum g between the two. The whole instrument is chromium-plated and may sterilized by boiling if necessary.

6. Assistants

In many respects the assistants are the most important factor in all anim work. One can improvise in matters of equipment but there is no substitu for the conscientious assistant unless one is prepared to undertake personal the whole management of the colony as well as the actual experimental pr cedures. Further references to the indispensability of the reliable assista are made in Chapters 5 (p. 96), 6 (p. 111) and 8 (p. 166).

The position of the animal-room assistant, as opposed to that of an assistant ngaged in chemical, bacteriological or histological work is not always satis- ctory. As indicated above, animal duties are too often regarded as a fatigue, hile research workers are sometimes to blame for appearing to set far greater ore by the accuracy of bench experiments than by that of the animal routine. rom a perusal of salary scales in force at different institutions one would not etect any obvious bar to the assistant who may wish, or be asked, to undertake sponsible animal duties, but in practice his or her promotion tends to be ow, a fact which must surely cause potentially first-class attendants to take another branch of work. The aptitude for animal management of the pains- king kind required in the laboratory may not coincide with examination ility, but really skilled animal assistants appear to be fewer in numbers than ose in almost any other branch of laboratory technology. A person qualified nd willing to undertake these arduous duties should surely receive every nancial and other encouragement. Success depends not only upon considera- on for animals and ability to handle them with ease but also upon great erseverance in observation, recording and the carrying out of menial tasks. any workers prefer women to men as animal assistants, but whoever is chosen e essential is absolute dependability coupled with genuine enthusiasm for the sk. Whether latent qualities of this kind develop into technological excellence ust in present circumstances depend largely upon individual tuition. There ould appear to be scope for regular courses of instruction in laboratory-animal sbandry.

7. The Infected-Animal Room

The precautions adopted at leading bacteriological laboratories, e.g. the ondon School of Hygiene and Tropical Medicine, the British Postgraduate edical School and the National Institute for Medical Research, are stringent the extreme. It is, of course, fallacious to attempt accurate work of this kind thout the most dependable technical assistance and efficient methods of ntrolling infection. The following recommendations are drawn from various urces and are intended only as a guide. They are impracticable without full tiseptic precautions and effective sterilizing equipment.

The *floors* should be asphalted and slope down gently to a gutter running der the windows, the gutter to be continuous with a similar gutter in other fected-animal rooms and to end at a drain provided with a fine-mesh grid to ld up particles of bedding, etc., which may be washed down to it. The *walls* ould be effectively waterproofed (that is, should stand violent hosing weekly th disinfectant fluids) up to a height of at least 6 ft. The *windows* should be -proofed in their opening parts with copper mesh. The *skylights*, if any, ould have the ventilating parts at the side, hinged at the top and opening twards. Fly-proofing then consists of permanently fixed frames con- ining copper mesh, and there is no interference with the light coming from e outside. Any cords or rods operating the skylights from the inside would ss through the fly-proofing and leave a hole for flies to get in. Spring *doors* e preferable, with large lever handles operated by the elbow. Special rubber ti-bang stops should be fitted. Panel *heating* is preferable to radiator heating less the radiator is so made that it has very few pockets for dirt and conse- ently for fly nests to accumulate in, and is capable of being hosed down

vigorously without undue rusting. Each room should have its own *sink* wit hot and cold taps of the surgeon's lever type for hand washing and a thir wide-bore tap to take a large-bore hose. The *hose* should reach to the farthes corner of the room and have a tap at its nozzle. *Wall brackets* should b supplied for animal-refuse buckets and for shovels and brushes so that thes may be hung out of the way when the rooms are being hosed down. Ther should be a special set consisting of shovel, stiff broom and mop for the anima rooms. The cage supports should be spaced so as to have a minimum numbe of bars (about four to the cage). The supports can be covered with removab sheets of galvanized iron if it is desired to put small cages on the racks.

A rigid routine must be adopted for the selection and handling of th animals, for the changing, cleaning and sterilization of cages, and for the contro of food, water and bedding. It must be firmly impressed upon all concerned whether senior or junior, that wholehearted co-operation is essential if costl errors are to be avoided. Only those who have specific reason or permissio should enter an infected-animal room and in some laboratories no one is allowe to enter without first putting on rubber overshoes, which are removed imme diately on leaving, or to handle animals and equipment without wearing rubber apron and rubber gloves.

It is essential that animals selected for experiment should have been observe carefully for a considerable preliminary period for any evidence of infection o ill-health. While on experiment they must be inspected several times daily Dead animals must be removed in a specially kept and sterilized vessel, wit due antiseptic precautions, while infected animals which are to be killed mus not be touched with the naked hand but gently removed to the lethal chambe or other place in which euthanasia is carried out, every care being taken t ensure that infective material is not spilt. There must be a rigid techniqu for *post-mortem* examinations with adequate sterilization of all instrument before and after use and incineration of the carcase and of any materia removed therefrom. The carcases and fragments of small animals may con veniently be wrapped in several thicknesses of newspaper for removal to th incinerator.

Clean cages to which infected animals are to be transferred should be s designed that contamination of their exterior is reduced to a minimum. No more than one infected and one clean cage should be open simultaneously Animals should be transferred swiftly and smoothly and any gloves or instru ments used to effect the transfer should be washed in disinfectant solution an rinsed with sterile water before the next transfer is made. Used cages shoul be sterilized as quickly as possible and on no account should the inside of th cage be touched beforehand. It is safer to sterilize cage and bedding and t dispose of the bedding later. Even so, the bedding should be incinerated wit the usual precautions in case infected material in its centre has escaped fu sterilization.

8. Some Notes on Technique and further sources of Information

It is beyond the scope of this book to go into details of **experimental techniqu** except in so far as they affect the well-being of the stock. Handling, marking anæsthesia and euthanasia are dealt with in this respect in the separate chapter on species. Among further sources of information are vol. 9 of the Medica

Research Council's *A System of Bacteriology* (Wilson Smith, 1931), vol. 9 of Abderhalden's (1929) *Handbuch der Biologischen Arbeitsmethoden*, and chapters in various text-books of bacteriology or pharmacology, e.g. Eyre (1930), Hollmann & Hanzlik (1939). Reference might be made also to Coward's (1938) *Biological Standardisation of the Vitamins*. There is a useful chapter on keeping experimental animals germ-free in Reynier's (1943) *Micrurgical and Germ-free Methods: Their Application to Experimental Biology and Medicine*.

The monograph on *The Rat in Laboratory Investigation*, edited by Griffith & Harris (1942), to which extended reference is made in Chapter 6, provides information which is of great value to all who are engaged in work upon the smaller laboratory animals. The small publication of Herrlein (1940), *A Practical Guide on the Care and Uses of Small Animals for Medical Research*, contains many useful points and well-arranged lists of references. The Soviet monograph *Razvedenie i soderzhănie melkikh laboratornykh zhĭvotnykh* [*Breeding and maintenance of small laboratory animals*], by Kovalevsky (1944), was recently received through the kindness of Prof. V. Soloviev of the Mission of the Union of Societies of the Red Cross and Red Crescent, of the U.S.S.R. in Great Britain. An English translation is not yet available but the work appears to provide useful information upon care, management and diseases. From the husbandry standpoint there is much practical information in the publication *Living Specimens in the School Laboratory*, to which reference is made in chapters 20 and 23, and in Ward & Fisher's (1939) *Animals as Friends and How to Keep Them*, while useful information is often to be found in popular publications such as those of Fur & Feather, Ltd., Idle, Bradford, and the Exchange, Bazaar & Mart, London. Brief but valuable notes on the management and feeding of many species are given in the paper by Walker (1942). The (1941) publication of the same author contains useful material on animal behaviour, a subject to which Russell's * (1938) text-book provides a valuable introduction for the scientific worker. (See also *Laboratory Manual for Experimental Comparative Psychology*, by Warden, Ross & Klein, 1942, and the paper by Edwards, 1945.)

Reference is made elsewhere in the Handbook to *The Care and Feeding of Laboratory Animals* (Ralston Purina Co., 1942). Campbell's (1928) thesis on *Growth, Reproduction and Longevity of Experimental Animals as Research Criteria in the Chemistry of Nutrition* and Coronel's (1937) small publication, *Guide for Raising Small Laboratory Animals* may also be worth consulting. References of nutritional importance are those of Dunlop (1933) and Slanetz (1943).

Important contributions which came to our notice too late to be sent on to individual authors are the papers presented at the Conference on "Animal Colony Maintenance" held on November 10 and 11, 1944, under the auspices of the New York Academy of Sciences (Section of Biology). These comprise:

"Genetic purity in animal colonies," by F. B. Hutt (Dept. Poultry Husbandry, Cornell University); "The mating of mammals," by Carl G. Hartman (Dept. Zoology and Physiology, Univ. Illinois); "Feeding laboratory animals," by J. K. Loosli (Cornell Univ.); "Infectious diseases of laboratory animals," by Herbert L. Ratcliffe (Dept.

* Dr. E. S. Russell is Past-President of the Institute for the Study of Animal Behaviour, an organization of interest to those who maintain stocks of laboratory animals. Details may be obtained from Dr. P. H. T. Hartley, Edward Grey Institute, 91, Banbury Road, Oxford, or from Prof. A. N. Worden, Cromwell House, Huntingdon.

Pathology, Univ. Pennsylvania); "Influences of environmental temperatures on warm blooded animals," by Clarence A. Mills (Dept. Experimental Medicine, Univ. Cincinatti) and "Financing and Budgeting," by C. N. W. Cumming & F. G. Carnochan (Carwort Farms Inc., New City, N.Y.). We are indebted to Dr. E. J. Farris for having forwarde copies of these papers which we should be prepared to loan on application if they are no available in published form in Great Britain by the time the Handbook is published.

As noted in the preface, we were fortunate in receiving certain informatio from the Soviet Union. This information arrived in the form of micro-film photostat copies of which have been made and are available for reference. there is sufficient demand English translations will be made available.

The Publications comprise *Laboratory Rats and Mice*, by P. P. Sacharov (1933), pul lished by the Soviet State Publishing House, Moscow (this 91 pp. illustrated paper contai sections on "The fundamental and biological properties of mice and rats," "Feeding mice and rats and avitaminosis," "The rearing of mice and rats," "Cages for mice an rats," "Diseases of mice and rats," and "Mice and rats as laboratory animals"); and series of shorter papers, mainly from the Soviet journal, *Laboratory Practice*, including "On the feeding of anthropoids in captivity," by P. V. Botchkarev & O. I. Nikolaev (1934); "The importance for medicine of the rearing of monkeys," by V. D. Shervinsk (1932); "On a cage for monkeys for laboratory experiments," by P. V. Botchkarev A. N. Smirnov (1934); "On cages for rabbits" (illustrated), by A. I. Metelkin (1931) "A new laboratory animal, the Transcaucasian hamster," by S. P. Kandelaki (1941) "Experiments on the maintenance of porpoises under natural conditions," by G. I Shubin (1941); "On the changing of water in tanks for aquatic animals," by V. I Sudarikov (1939); "Baths for the maintenance of normal body temperature in narcotise animals," by P. M. Subbotin (1939); "The feeding of axolotls," by A. Peredelsky (1941) and "The control of bugs (*Cimex lectularius*) in animal houses," by A. I. Metelkin (1938).

For diseases of laboratory animals further reference may be made t Jaffe's (1931) *Anatomie und Pathologie der Spontanerkrankungen der kleine Laboratoriumstiere, &c.*, to McIntosh (1931), and to Ratcliffe's paper noted abov Other general publications on disease include the paper of Hardenburg (1929) and an article on "Bacteriology and control of acute infections in laborator animals" appearing in 1914 in the *Journal of Pathology and Bacteriology*, 1 (No. 4), pp. 445–455.

Most developments in laboratory-animal technique are noted in one o more of the abstracting journals listed at the end of Chapter 8, p. 176. *Scienc Nature, Lond.* and the *Journal of Laboratory and Clinical Medicine* are amon the periodicals in which original publications are most often found. For tech nicians there is now an abstracting service in the *Monthly Journal* of th Institute of Laboratory Medical Technology.

Anæsthesia. Most of the chapters on species contain details of metho of anæsthesia and euthanasia. Where such information is not given referenc should be made to the corresponding chapter of a similar species (e.g. t Chapters 6 and 8 in the case of the other murine rodents). Ferrets are usuall etherized although there seems no obvious reason, given suitable restrain why basal narcotics should not be employed. A useful table of anæsthet doses of tribromethanol (avertin) for three species is given by Dr. G. Brownle on p. 129. For the anæsthetization of pigeons, see the textbooks quoted i the notes on poultry in Chapter 23. Wright (1947, pp. 203–209) has summarize much of the relevant information appearing in this textbook.

Baker (1945) notes that it is sometimes stated that it is undesirable to ki animals by anæsthetics when tissues are required for histological or cytologic purposes, especially in studies of lipoid cell-components, but suggests that th statement does not rest on experimental proof. He maintains that whi

her anæsthesia prolonged for a period of hours causes histological changes in ıe nervous system (A. Weil, *Pflügers Arch.*, **223**, p. 351, 1930) there is no roof that the use of anæsthetics for the period necessary to kill an animal roduces effects that can be demonstrated by microscopical studies of the ssues. In the present state of knowledge he suggests is reasonable to kill animals by anæsthetics in stological and cytological work, except when the ng itself is being studied. For a further comment ı the effect of anæsthetics upon the tissues, see the ction on anæsthesia and euthanasia in Chapter 4, p. 74–80.

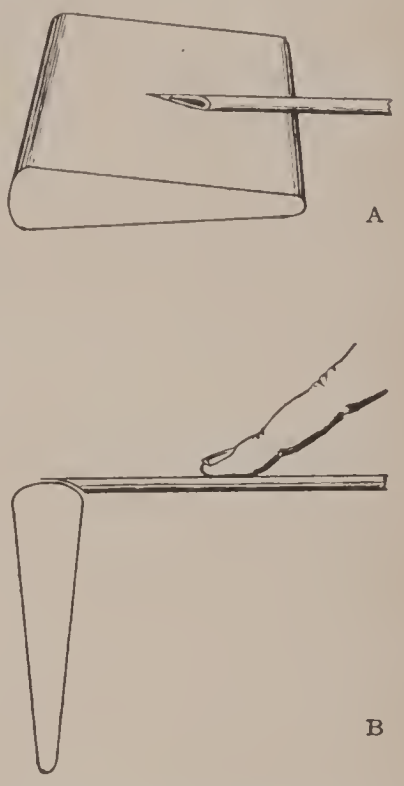


Fig. 17.—Method of Sharpening Hypodermic Needles.

The sharpening of hypodermic needles. We are debted to Colonel Leonard Colebrook, R.A.M.C. Colebrook, 1945) for the following note on the harpening of hypodermic needles. The apparatus quired is a small Arkansas slip-stone, 3 to 4 in. long nd with one curved and two flat surfaces, and a mple hand lens of magnification ×6 or × 8. The eedle is examined with the hand lens to see what rrection, if any, is required. If it lacks a fine point ch side is rubbed in turn on the flat surface of the one, moistened with water or saliva or oil (Fig. 17A), e effect being watched from time to time with the and lens. When a sharp point has been produced, e bevelled surface of the needle is pressed lightly ı the curved surface of the stone and rubbed until is surface of the needle is smooth (Fig. 17B). nally, the lateral edges of the bevelled surface are amined with the hand lens and, if necessary, bbed until the whole needle forms a sharp point with no roughness of the tting edges anywhere.

9. Units of Length, Weight, Volume and Temperature

The units used in the different chapters of this handbook are not expressed niformly. For convenience the following approximate equivalents are ven:

Length (*and area*).

1 inch (in.)	= 2·54 centimetres (cm.)
1 cm.	= 0·394 in.
1 foot (ft.)	= 30·48 cm.
1 square inch (sq. in.)	= 6·45 sq. cm.
1 sq. cm.	= 0·155 sq. in.

Weight.

1 ounce (oz.)	= 28·375 grammes (g.)
1 g.	= 0·0352 oz.
1 pound (lb.)	= 454 g.
1 kilogramme (kg.)	= 2·20 lb.
1 grain (gr.)	= 0·065 g.

Volume.

1 millilitre (ml.) or cubic centimetre (cm.)	= 0·0610 cu. in.
1 cu. in.	= 16·4 ml. or c.c.

Temperature.

To convert ° C. to ° F., multiply by 1·8 ($\frac{9}{5}$) and add 32, e.g. 50° C. = 122° F.

To convert ° F. to ° C., subtract 32 and multiply by 0·55 ($\frac{5}{9}$), e.g. 98° F. = 36·6° C.

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Notes added in proof, December 1946.—(1) A general review of the methods of feed and breeding laboratory animals has been undertaken at the National Institute Medical Research and a series of papers designed to record conclusions of general inter has begun to appear in the *Journal of Hygiene* under the title of " Feeding and breed of laboratory animals." In the first (" Rat and mouse cubes, and cube containers A. S. PARKES (*J. Hyg., Camb.*, 1946, **44**, 491–500) describes a cubed diet suitable growing and stock rats and mice, together with wire-mesh containers adapted to vari types of cage, and records the consumption and requirement of these cubes by growi rats and mice. In the second (" Growth and maintenance of rabbits, without fr green food ") H. M. BRUCE & A. S. PARKES (*Ibid.* **44**, 501–507) state that fresh greenst is not necessary for rabbits, provided drinking-water is supplied, and give details of a diet which has given very satisfactory results, especially in pellet form, when supplement with hay and water.

(2) The Biological Methods Group of the Society of Public Analysts proposes to h meetings during 1947 for a symposium on the production and care of laboratory anima The Hon. Secretary of the Group is Dr. Eric C. Wood, whose address is given on t page, and from whom details of publication of proceedings may be obtained

(3) A technical communication (No. 16) entitled *Diet in relation to reproduction a the viability of the young. Part 1. Rats and other laboratory animals*, dated August 19 is being published by the Imperial Bureau of Animal Nutrition, Aberdeen, at 3s. 0d., b copies were not yet available at the time of going to press.

CHAPTER 3

PESTS OF THE ANIMAL HOUSE AND THEIR CONTROL

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1. General considerations. 2. The bedbug. 3. Fleas. 4. Lice. 5. Mites and ticks. 6. Flies and mosquitoes. 7. Cockroaches. 8. Insect pests of stored foodstuffs. 9. Rats and mice.

1. General Considerations

Insect pests are unnecessarily common in animal houses and their incidence uld be very greatly reduced without much difficulty. In order to reduce it ıe must have a working knowledge of their life histories : these are given in is chapter, which is concluded by a list of references for further reading. ıe ease of controlling such pests depends very much on the way in which the ımal house and its fittings are made, and this account should be studied in nnection with chapter 2, which deals with walls, floors, cages, racks, etc. eneral cleanliness, which means the use of good bins for bran, grains, etc., d thorough and frequent disposal of bedding and excrement, is very ıportant.

A general point requiring emphasis is that many laboratories buy their ımals from small dealers, who breed them, perhaps, under very unhygienic nditions. The incoming stock may be lousy, mangy or flea-ridden ; or the avelling-boxes may contain bugs in cracks in the woodwork. There should, erefore, be a separate room for unpacking, inspection and quarantining ; avelling-boxes should not be carried into the animal house.

Insect pests are harmful in several different ways :

(1) The blood-suckers (e.g. bedbugs, fleas, sucking lice, certain mites) use loss of blood and much irritation and scratching.

(2) Certain mites cause mange and other chronic diseases of skin, hair or athers.

(3) A number of parasites (bacteria, viruses, protozoa, nematodes, cestodes) ust pass through an insect host and cannot go direct from mammal to mammal. ıey are generally controlled by attacking the insect.

(4) There are moths and beetles (adults and larvæ) and some mites which ntaminate and destroy cereal and other foodstuffs.

A brief note on the zoological position of these pests may, perhaps, be helpful. xcluding the rats and mice (which are dealt with in this chapter solely on ounds of convenience), the pests are either insects or arachnids. They are stinguished as follows :

Insects have the trunk divided into three regions (head, thorax and abdo- en), three pairs of legs ; some have wings (one or two pairs). All lay eggs.

In some insects (e.g. bugs) the egg hatches producing a little edition of the paren i.e. a small bug. In others (e.g. flies, moths) the egg produces a maggot caterpillar, which feeds and grows, passes into a resting stage (pupa) and the hatches into an adult fly or moth. Insect pests include bugs, fleas, mosquito lice, beetles, moths.

Arachnida. The only Arachnida which are pests are the *mites and tick* not at all typical of the group. In these the trunk is not divided into regio and they have four pairs of legs. They are all wingless. The egg produces larval mite, like its parent in shape, but with three pairs of legs.

2. The Bedbug

Occurrence. The bedbug (*Cimex lectularius*) is a common pest of anim houses. It could easily be eradicated if cages and houses were proper constructed, and the insect's natural history understood, even without recour to modern insecticides.

Biology. Bedbugs are brown, flat and wingless. The outline is oval ar the full-grown bug about 4 mm. long, 3 mm. broad. The young bugs (nymp or larvæ) are like the adult in shape, but smaller.

Bugs live nearly their whole life in small crannies. In an animal hou they find harbourage in cracks in the structure of cages, or the racks especial if wooden ; in cavities in walls, as where mortar is decayed, or whitewa flaking off ; and in nail holes, and behind such fixtures as switchplates boards which cover wiring. In a properly constructed animal house with goo racks and metal cages (see pp. 21–38) these harbourages are much reduce but bugs may still be abundant, for instance in the crack where the cage fi into its tray. The bugs themselves are often overlooked, as they are nocturn and during daylight are hidden away in crevices. Spots of their characteris brown excrement just outside the hole in which they harbour are evidence th bugs are or have been present.

It seems that bugs are capable of walking considerable distances from t crack in which they habitually dwell to the man or animal on which they fee They run actively at night, and can move about even if the temperature below 10° C. They may also be carried about in crates and boxes in whi dealers send animals. Such boxes should be regarded with suspicion an opened in a quarantine room ; they should never come into the animal hou itself, and animals should not be kept in them after arrival.

Bugs generally suck blood at night. Feeding only occupies a few minute but a very large meal of blood (often exceeding the weight of the bug) is take The bug cannot take any food except the blood of mammals or birds.

The commonest host is man, but the bedbug feeds readily on rabbits, mic rats, hens, etc., and can live and multiply indefinitely on many host specie Natural infestations occur in chicken houses, rabbit hutches, and anim houses. Pigeons are attacked by a very closely related pest, *Cimex collu barius*.

As in other insects, the sexes are separate. Both feed on blood, and aft pairing the female lays eggs and then feeds and lays eggs repeatedly. The ra of egg-production depends mainly on temperature and chances of feeding ; f instance at 23° C. if a female is fed once or twice a week she will maintain a output of 5 to 10 eggs weekly for two to five months. The egg is an ov

pearly object and is laid in small cracks, under loose wall-paper, etc., where it s fixed by a sticky substance which hardens quickly. At one end is a lid, vhich is pushed up by the young nymph (larva) at the end of incubation. The nymphs resemble adults but are much smaller. They feed on blood, moult he skin, become larger, and feed again. There are in all five moults, i.e. five ymph stages. At the last moult the bug becomes an adult male or female.

Assuming that the insect can feed whenever it requires to do so the length f the life-cycle is dependent mainly on temperature. The following figures ive the approximate period for one complete generation :

° C.	° F.	days.
15	59	237
18	64	125
23	73	62
28	82	34

Below 15° C. the bug can move about and feed, but larvæ cannot moult their kins and eggs do not hatch, so that continuous existence is not possible. If edbugs are starved they are capable of living very long periods. Their urvival depends on a variety of factors. The period is longest at about 3° C., at a high humidity, and if the bugs have been well fed and are com-letely undisturbed. The following numbers of days (for mated bugs, at very igh humidity) are, perhaps, seldom attained in nature :

° C.	° F.	Male.		Female.	
		mean.	max.	mean.	max.
7	44	220	386	286	465
13	55	338	470	360	565
18	64	152	260	143	225
23	73	85	136	69	127

t will be seen that the maximum period may exceed one year, but does not each two.

The increase of a community of bugs depends on many factors, temperature nd opportunity to feed being the principal. There are reasons for thinking hat in an unheated bedroom in London the bugs are greatly reduced by the rolonged low winter temperature, for they would not feed or lay eggs from ovember to April ; the summer would permit a 20-fold increase, but the ext winter would reduce the numbers almost to the original low figure. With entral heating, on the other hand, not only is the possible increase greater, but he winter does not cut the population down, so that a very great increase might ccur in a second or subsequent year. The causes of death are hardly known. n infested animal houses the experimental rats and mice eat many bugs and aintain some measure of control. There is no truth in the statement that ockroaches eat bugs.

The bedbug (*Cimex lectularius*) inhabits houses, etc., in practically all parts the world. In the tropics a second species, the tropical bedbug (*C. rotun-atus*, or *hemipterus*) also occurs. The habits of the two are very similar.

In the laboratory one can infect bugs with a considerable variety of patho-enic micro-organisms (spirochaetes, bacteria, Leishmania), and some of these an be recovered alive a considerable time later. There is no evidence that in ature the bug acquires these parasites, or can transmit them.

Control. In the prevention and control of bedbugs, the harbourages

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available to the insects are of cardinal importance (see above). The bug stay in hiding during the daytime so that a large colony may develop wit many eggs laid before the infestation is detected. Moreover it is much easie to prevent bug infestation in a room with relatively few crevices and hidin places. Some harbourages can be avoided in the construction and furnishin of new animal houses, e.g. by using metal cages and fittings instead of woo wherever possible. In existing houses it is important to avoid providin additional cover for the insects by leaving rubbish about.

If an infestation of bugs has been detected, cages and boxes which have lon remained undisturbed must be moved, and the floor and shelves swept an scrubbed. The opportunity should be taken to discard any lumber whic may have accumulated. During the cleaning operations the location an extent of the infestation will be ascertained. The most likely places to b infested are the animal cages themselves (especially in cracks and joints i they are of wood) and crevices in the adjoining walls. If the infestation is no heavy, it may be eliminated by a thorough cleaning supplemented by dabbin the focal points with a paintbrush dipped in such an insecticide as 10 per cen Lethane 384 in kerosene.

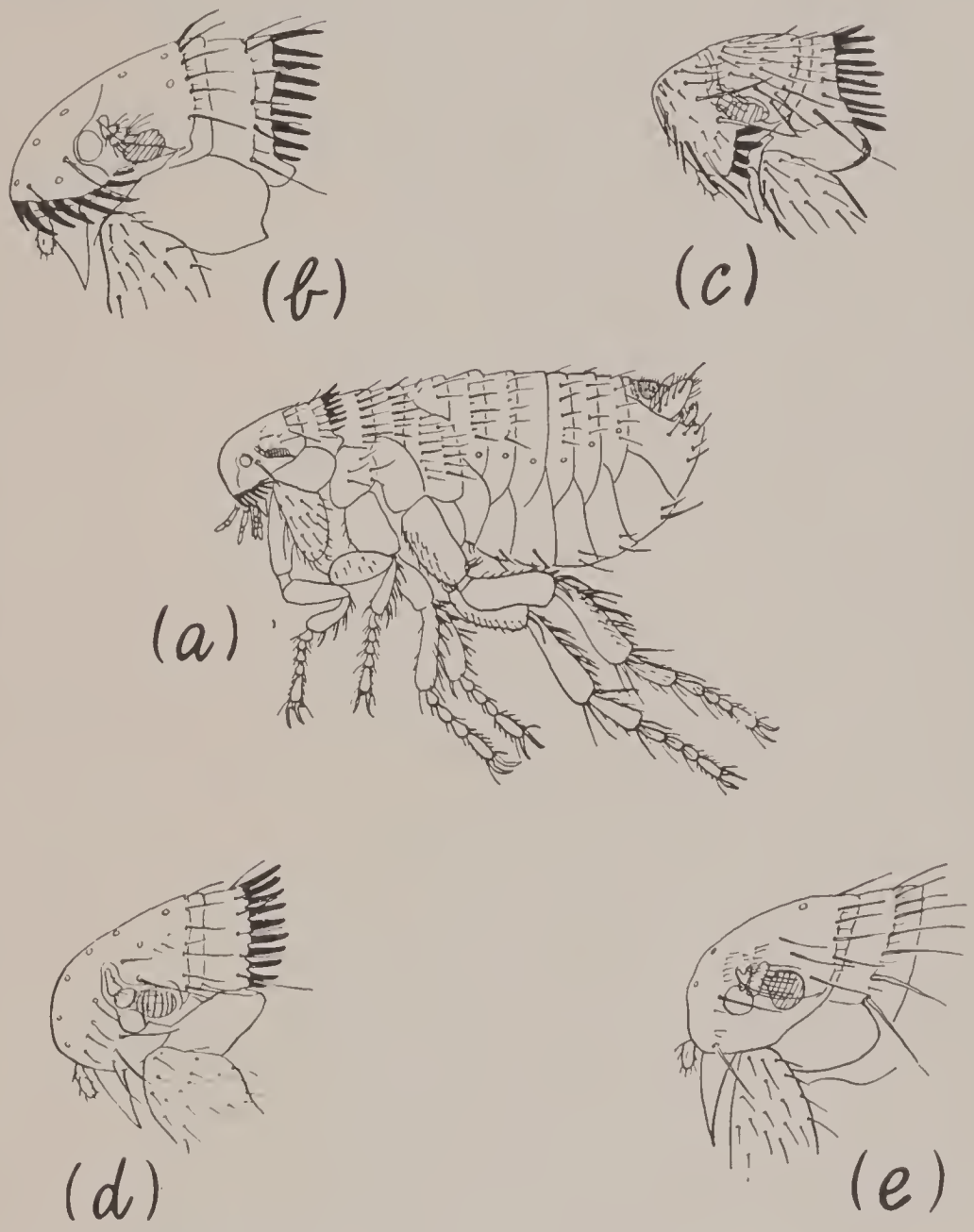
With a heavy infestation, in a room containing numerous harbourage more drastic measures may be necessary. The only measure which can usuall destroy the whole population of bugs at once is fumigation. But this entail removing all the animals for at least a day, even two days with hydroge cyanide. Probably the most effective simple measure is to spray walls an cages with a persistent insecticide. About the most effective is a 5-per-cen solution of D.D.T. (dichlor-diphenyl-trichlorethane) in odourless kerosen Spraying is best done with a knapsack sprayer or a power paint-sprayer wit the nozzle adjusted to give coarse drops. If these are not available a han spray-gun can be used ; but it will be found very hard work to do the treatmen thoroughly. Special attention must be paid to all crevices where bugs may b hiding ; they should be flooded with insecticide by opposing the nozzle of th sprayer to the openings. The general wall surface should be covered with film of about 5 c.c. ($\frac{1}{6}$ oz.) per square foot. This insecticide is rather slow i acting upon bugs, so that live insects may be observed for 24 or 36 hours afte spraying. Nevertheless, all insects contaminated with it will eventually die and the lethal effect of the film on sprayed materials lasts for several months Used in this way the material is harmless to mammals and birds.

3. Fleas.

Effects of infestation. In the adult stage all fleas are blood-suckers o mammals or birds. The various species of flea each has its own particula host which it prefers to bite, but this preference is not rigid. Thus the fleas o many animals and birds will bite man more or less readily, and conversely th human flea (*Pulex irritans*) will feed on the dog, the pig and other animals.

Apart from the irritation caused by their bites, fleas are obnoxious becaus they are the vectors of certain parasitic worms and other infections. Normall these are passed from one animal to another of the same kind. But sinc fleas are not confined to a single host, the disease or parasite may be conveye to man or to other animals. Thus plague, normally an infection of rat conveyed by the bites of fleas (*Xenopsylla cheopis* and others), may be trans

ferred to man. Murine typhus is another disease mainly transmitted by rodent fleas (*Xenopsylla, Nosopsyllus* and *Leptopsylla*), either from rat to rat, or rat to man. Fleas can also transmit from rat to rat the protozoan *Trypanosoma lewisi*.



IG. 18.—(A) *Ctenocephalides canis*—THE DOG FLEA (Male). (B) HEAD OF *Ctenocephalides felis*—THE CAT FLEA (Female). (C) HEAD OF *Leptosylla segnis*—THE MOUSE FLEA (Female). (D) HEAD OF *Nosopsyllus fasciatus*—THE RAT FLEA (Female). (E) HEAD OF *Xenopsylla cheopis*—THE TROPICAL RAT FLEA (Female).

[*Redrawn from "Insects of Medical Importance," by J. Smart, K. Jordan and R. J. Whittick. By the courtesy of the Director of the British Museum, Natural History.*]

A number of tapeworms (Cestodes) are carried by fleas which act as intermediate hosts. The eggs of the tapeworm, in the fæces of the mammal, are swallowed by flea larvæ. Inside the flea the cestode develops into the "cystercercoid" stage. Return to the vertebrate intestine is accomplished

when a parasitized flea is swallowed. The best known cycle of this type passed by the worm *Dipylidium caninum*. The main host is the dog (son times the cat or man) and the intermediate stage is passed in the flea *Cter cephalides*. The rat and mouse tapeworms, *Hymenolepis spp.* are also said be transmitted by rodent fleas (*Xenopsylla*, *Nosopsyllus*, etc.) but these wor are able to pass direct even if no fleas are present.

Life-history. In order to control fleas or to eliminate them it is necessa to know something of their biology.

After mating, female fleas lay their eggs more or less at random. Many them are placed in the fur or plumage of the host and eventually fall to t ground, often near the host's sleeping place. The eggs develop into tir white, maggot-like larvæ, which live among dirt and under rubbish, and whi are quite unlike the fleas themselves. These larvæ feed on miscellaneo organic debris and especially on the partly digested blood in the excrement adult fleas. After a certain time, which depends on the temperature and the kind of flea, the larva spins itself a cocoon inside which it becames a chrysa or pupa. After an interval the pupa develops into an adult flea and the l cycle is completed. The whole process from egg to adult may take only 3 o weeks in summer, very much longer in winter. The adult flea does not emer from the cocoon at once, but may remain quiescent for an indefinite perio It is stimulated to emerge if the neighbourhood is shaken, for instance by t rat or other host returning to its nest.

Adult fleas live on blood which they suck by puncturing the skin of t bird or mammal. Though all can suck blood, it seems possible that so species may be able to lay eggs without doing so.

In a warm environment, with opportunity to feed at will, the adult life many sorts of fleas runs its course in 3 to 4 months. At low temperatur however, the adult life may be spun out to a year or so. The length of ti that fleas can survive without a blood meal depends largely upon their enviro ment. At a warm temperature (say 25° C. or over) they starve to death in week or two. But under cool, moist conditions (below 13° C. and abo 80 per cent. relative humidity) some species at least can survive several mont or even a year. The adults and larvæ of *Xenopsylla cheopis* are killed by exposure of one hour to 41° C. There is a high mortality among flea lar below about 60 per cent. relative humidity, and in very dry air they soon d However, flea larvæ living under rubbish may be in a micro-climate where t humidity is much higher than in the free air. Undisturbed dirt and rubbis therefore, provide flea larvæ with essential shelter as well as food.

Control. To eradicate fleas, two sorts of measure are necessary : the ad fleas on the animal's body must be killed and the young stages in the breedi sites must be extirpated.

To destroy fleas on animals, dust a powdered insecticide among the ro of the fur or feathers. A very effective dust is made from powdered der diluted with a mineral powder, so as to contain 1 per cent. of rotenone. milar powder made from powdered pyrethrum, to contain 0·5 per cent. ethrins, is equally good, and so is a dust containing 5 per cent. of D.D. (or diphenyl trichlorethane). Flowers of sulphur have been reco mended for fleas on chickens, but sulphur is not generally considered to be powerful insecticide. Whatever is used it must be finely ground so that it will adhere well. Animals tend to lick themselves, and this rules out certa

naterials which are insecticidal but would be poisonous if swallowed by the ost.

Destruction of the young stages of the flea should be preceded by a cleaning f the cages and of the animal rooms generally. All combustible rubbish should e burnt. The bottoms of the cages and the floor should then be swabbed ver with water containing 4 per cent. of Lysol, or with a paraffin-oil emulsion nade up as follows : dissolve 3 parts of soft soap completely in 15 parts of ot water ; then add gradually 70 to 100 parts of paraffin (kerosene) with much tirring or shaking. The final emulsion should be white and creamy with no ree oil. Dilute it before use with 20 parts of water.

If there are any inaccessible places where fleas might breed and which cannot e cleaned as described, flake naphthalene should be scattered in them.

4. Lice.

The word " louse " includes several sorts of small insects not closely elated to one another, e.g. :

(1) Blood-sucking lice (Anoplura), which pierce skin and take blood from apillaries ; lice of this group occur only on mammals.

(2) Mallophaga, which bite off solid particles of scurf, epidermis or feather. Most are found on birds, but certain types are characteristic of mammals. There is no general English word for Mallophaga ; " bird louse " or " feather ouse " is misleading, as some occur on mammals.

Lice of both these types are rather similar in appearance and in biology, iving all their life on the surface of mammal or bird, cementing eggs to feathers r hair, and tending to be specific to one species of host. If the insects become eparated from their host they starve to death as a rule in a few days.

Several blood-sucking lice (Anoplura) occur among the small laboratory nimals. The rat is very frequently infested with its louse (*Polyplax spinulosa*), which also occurs commonly on wild brown rats (*Rattus norvegicus*) and black ats (*R. rattus*). In the animal-house these insects tend to be particularly bundant on animals which are in poor condition or chronic ill-health, including nimals on deficient diets or exposed to the chronic effects of certain drugs. his louse transmits the *Rickettsia* of murine typhus from rat to rat. The ouse of mice *Polyplax serrata* also is common in animals. Other blood-ucking lice occur on the rabbit and ferret. Mallophaga occur on the domestic owl (several species), and on ducks, pigeons and canaries. The guinea-pig the host of four different sorts of Mallophaga and, curiously enough, of no lood-sucking lice.

The simplest effective method of controlling these parasites is to apply a nely powdered insecticide to all the affected animals. The fur or feathers re ruffled back and the insecticide is dusted in among the roots. Powdered erris root, containing 1 per cent. of rotenone, is one of the most effective pre-arations for this use but it sometimes causes injury or even death to small nimals such as canaries or young rats. Animals weakened by deficient diets re similarly susceptible and in all such cases pyrethrum powder (0·5 per cent. more pyrethrins) should be used instead. Alternatively the synthetic secticide D.D.T. (dichlor diphenyl trichlorethane) used at 5 per cent. in a ineral diluent would almost certainly be efficacious and harmless. At the

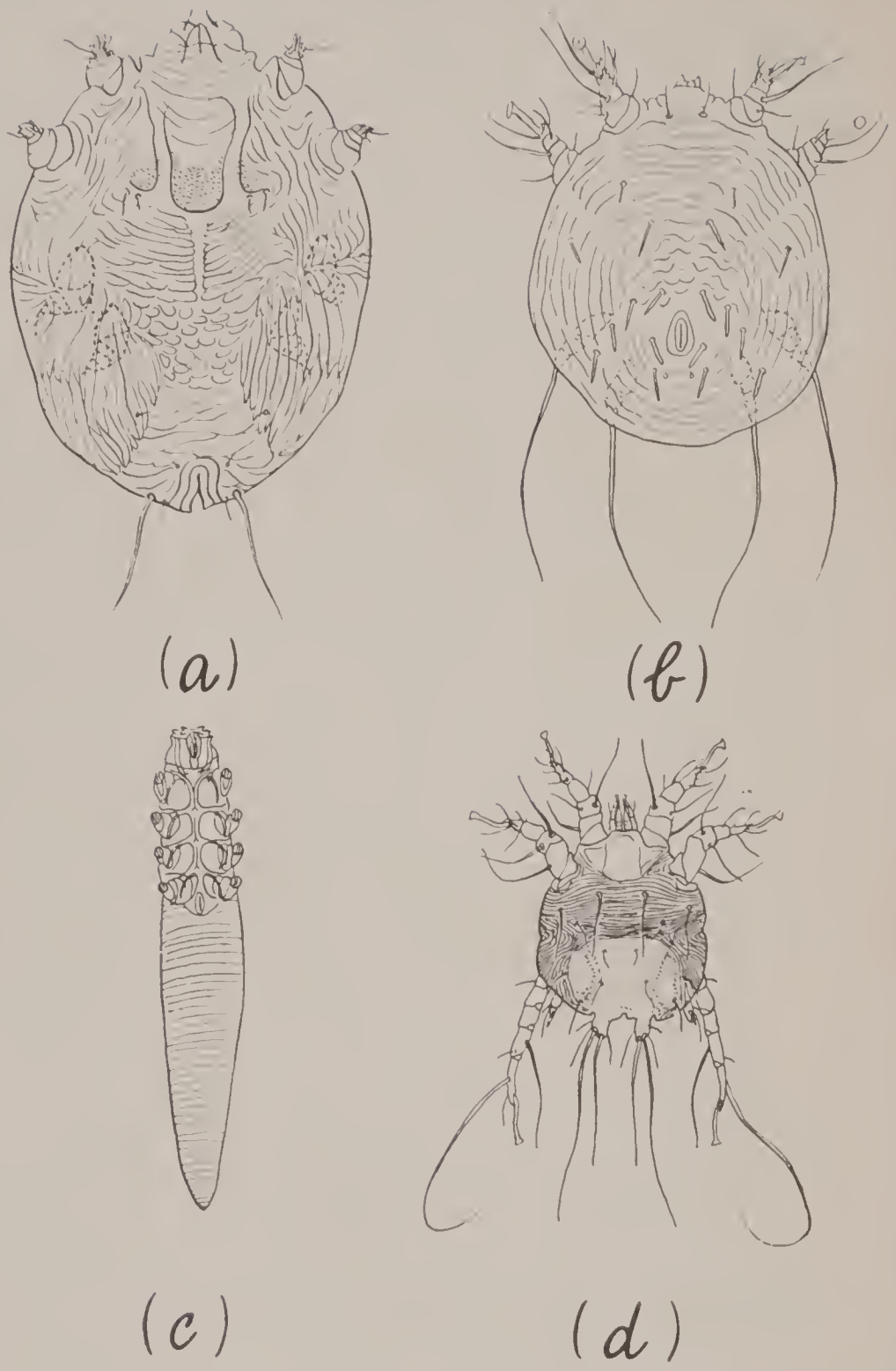


FIG. 19.—(A) THE MITE OF THE SCALY LEG IN FOWLS (*Cnemidocoptes mutans*). Dor
view of female. (B) *Notoedres cati*. Dorsal view of female. (C) *Demodex ca*
Ventral view of female. (D) *Psoroptes communis*. Dorsal view of female.

[*Redrawn from "Mites Injurious to Domestic Animals," by S. Hirst. By the courte*
of the Director of the British Museum, Natural History.]

me time as the animals are treated it would be a good plan to clean out the ges, dust a little insecticide into the corners and supply clean straw.

5. Mites and Ticks

Mange mites. There are a large number of mites (of the family *Sarcoptidae*) hich attack epidermal structures ; on mammals some live on the skin, causing perficial irritation ; others enter skin or its glands and cause some of the nditions known as mange or scab ; related mites cause analogous conditions the feathers and tarsal scales of birds.

Rabbits suffer from a variety of Sarcoptid parasites. The non-burrowing *soroptes communis* var. *cuniculi* may occur on various parts of the body, but commonest in the auditory meatus, producing large flakes of dead epidermis which the mites are abundant : its infectiousness seems slight, for it often es not spread from mother to young, or from one ear to the other. There e also two types of mange, due to burrowing mites, a *Notoedres*, causing a usty lesion, often round the eyes, and a *Sarcoptes* which appears to be com- onest on the nose. Heavy attacks may be fatal.

In rats, tame and wild, and in hamsters *Notoedres* is frequent on many parts the body, particularly the ear, and the ano-genital region.

In poultry *Cnemidocoptes mutans* causes "scaly leg," multiplying and using a serous exudate, which may be very thick and nodular, under the ales on the legs. A related species (*C. laevis*) causes depluming itch of geons and poultry ; it lives in the skin surrounding the base of feathers ; the rd then plucks at the feathers which become broken, little but the midrib of e feather being left in extreme cases.

A number of other mites occur among fur or feathers, not necessarily using any injury. Some of them are known to be predatory on the harmful tes.

The vermiform mites of the genus *Demodex* live in hair follicles and sebaceous nds of mammals. Some of the species are common in the skin of dogs, pigs d other animals suffering from a particular type of mange, though it is not ablished that the mite causes the mange. Species have been described m mice, rabbits, hedgehogs, etc.

The key to control of mange mites, and related parasites of birds, is the fact at the whole life-history is passed on or in the skin, or in masses of epithelium d serous exudate on which these animals appear to feed. The life history of st of them is not known in detail, but there are no indications that they can e for a long time away from the host. Moreover most of them are specific one species of host. Control should, therefore, centre on isolating the infected imal and its contacts.

The lesions should be treated with either :

(1) Sulphur ointment, which is successful on such bare spots as the fowl's or rat's tail.

(2) Benzyl benzoate emulsions, applied with a brush.

(3) Tetmosol, i.e. a solution of tetraethyl thiuram monosulphide, well bed in. The first two materials are available from pharmacists, for they e used in the treatment of human scabies ; Tetmosol is obtainable from perial Chemicals (Pharmaceuticals), Ltd.

Against scaly leg of fowls the dipping of the legs of the fowl in crude paraffin

has been recommended, or painting legs and perches with anthracene oil. T
treatment of fowls attacked by depluming mite, dipping of the whole bird i
sulphur bath ($\frac{1}{2}$-oz. soap and 2 oz. finely ground sulphur in 1 American gallon
water) is commonly used.

Blood-sucking mites. Certain mites of the family Parasitidae (Gemasid
suck blood of mammals and birds. *Liponyssus bacoti* normally feeds on ra
The adult mite takes a full meal of blood, leaves the rat and goes to rest i
crack in soil or building; it lays a small number of eggs, and then probal
takes blood meals laying batches of eggs after each. The egg hatches to produ
a larva which takes a blood meal, returning to a crack to moult its skin a
become a nymph; the nymph behaves similarly, finding a host, feedi
retreating and moulting to become an adult. The whole cycle may be pass
through in as short a time as 12 days. The same species of mite will feed
only on rats but on several species of rodents. It will also bite man, if he
living in close association with black rats; the bite is extremely irritating, a
on many people continues to cause annoyance for a number of days. The m
has an extremely wide distribution in temperate and tropical countries.
has been claimed, as a result of the work of Dove and Shelmire in Texas, t
this mite transmits the virus of tropical typhus. Transmission is by bite, a
the infection is hereditary in the mite.

Several other species of *Liponyssus* feed on poultry, sparrows, etc., and m
bite human beings. Birds are also attacked by other genera of blood-suck
mites. *Dermanyssus gallinae* is an important pest of domestic hens in te
perate climates and feeds on many other sorts of birds. It lives in nests,
cracks of fowl houses, coming out and sucking blood at night; it often bi
man if deprived of its normal food.

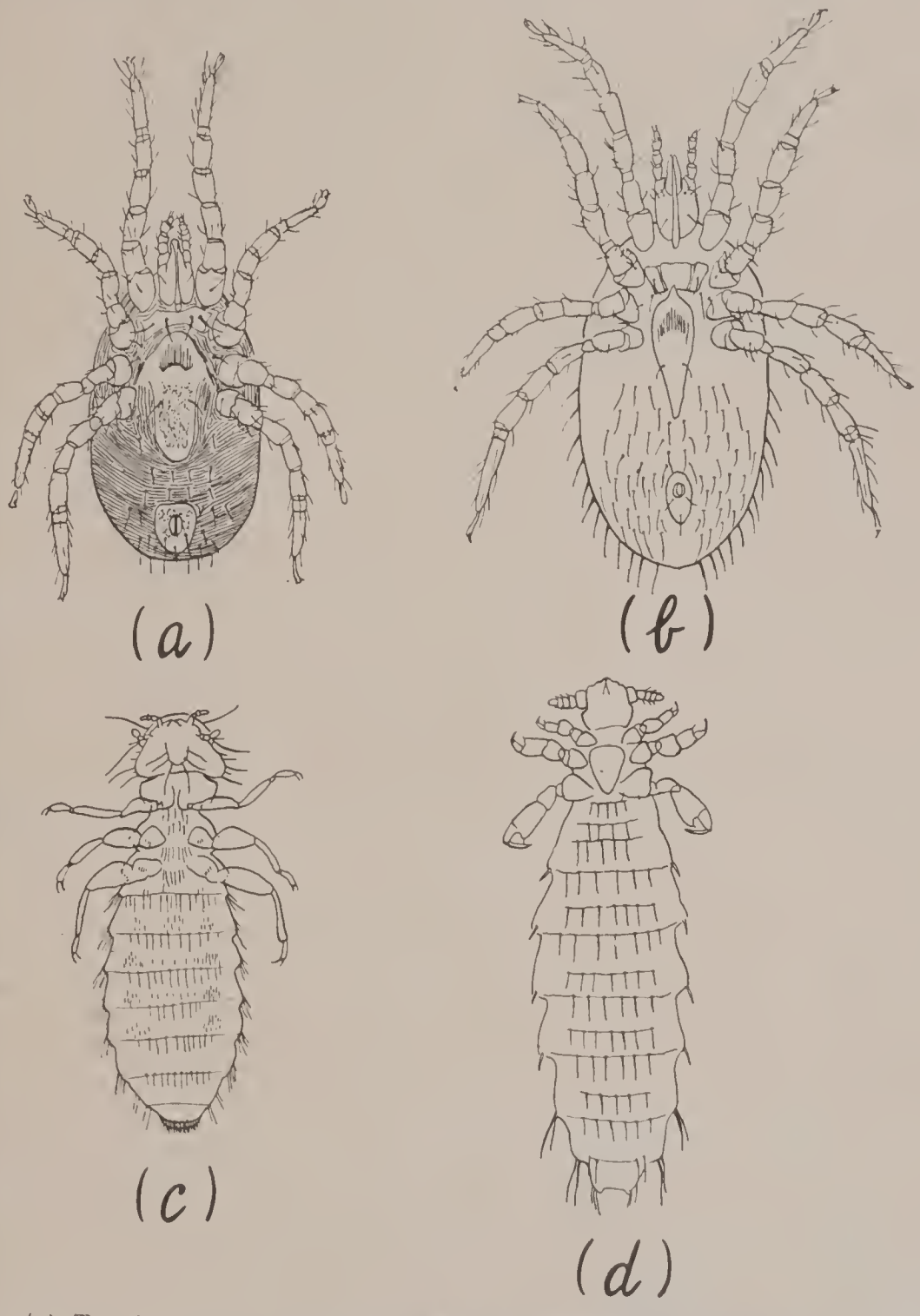
The control of *Liponyssus* and similar mites is by frequent destruction
the bedding of the rats or other animals, and reduction of cracks in cages
walls. No information exists on the effect of insecticides on these mites
eggs. Against *Dermanyssus* spraying of cages, perches, etc., with anthrac
oil, crude mineral oils, or creosote has been recommended. The painting
perches and woodwork with crude nicotine sulphate is effective; it must
remembered that the nicotine is dangerous to birds and mammals, so t
thorough ventilation is requisite, and the operator should not allow the mate
to remain on his hands. Fresh lime-wash destroys nicotine. Metal ca
may be flamed and protected from invasion by being put on tables the legs
which are set in vessels of water.

Ticks. Ticks are hardly pests of the animal house, at least in Great Brita
ticks (*Ixodes*) are, however, common on newly caught wild hedgehogs. Th
may be destroyed by touching them with a derris emulsion or an arseni
cattle dip, applied with a small brush. The hedgehog is often infested w
fleas as well as ticks: a derris dust containing 1 per cent. of rotenone w
destroy both pests.

6. Flies and Mosquitoes

Flies. The larvæ of many flies feed on decaying organic matter, and
smell of rotting substances attracts the females, who come to lay eggs. T
house-fly (*Musca domestica*) is particularly attracted by the smell of anim

crement. Since this odour is to some extent unavoidable in animal houses, house-fly is likely to be troublesome in them. The presence of numerous use-flies is objectionable for several reasons: they irritate man and beast l, by alternately visiting food and fæces, they transmit germs. In a



. 20.—(A) The Blood-sucking Fowl Mite (*Dermanyssus gallinae*). Ventral view of female. (B) The Blood-sucking Rat Mite (*Liponyssus bacoti*). Ventral aspect. (C) The Biting Louse of Fowls (*Eomenacanthus stramineus*). (D) The Rat Louse (*Polyplax spinulosa*).

[(A) *and* (B) *have been redrawn from "Mites Injurious to Domestic Animals," by S. st. By the courtesy of the Director of the British Museum, Natural History.*]

carelessly kept animal house flies may breed in the dung or rubbish. T development time is quite short, so that a fresh generation may appear in al three weeks in the British summer. This can and ought to be prevented regular cleaning of the animal cages every one or two days. The dung refuse should be incinerated. If this is neglected the flies will becom nuisance not only in the animal houses but in adjacent buildings. The that animals are often kept in the grounds of hospitals makes this n important.

Even if an animal house is kept reasonably clean, however, there inevitably be sufficient odour to attract flies in summer. Screening the ro (see p. 41) with wire gauze will keep them out, but is expensive. M can be done to reduce flies by the regular use of fly traps and papers, and la numbers can be killed by an easily-made poison bait. Make up 1 to 2 per c formaldehyde in milk or else in water with a trace of sodium bicarbonate or li water to neutralize any formic acid which may be present. Pour out a sı quantity of solution into a few open saucers in each room every day.

Control of flies over a long period can be achieved by spraying all t resting places (walls, cages, hanging lamps and fittings) with a solutio D.D.T. in kerosene, as described for bed bugs (see p. 52). This treatm renders the walls lethal to any flies which settle on them for many we The action of the drug is not very rapid but it should take effect in an h or so.

Apart from the house-fly, small flies of the genus *Drosophila* may attracted to animal houses and breed in pieces of decaying vegetation from food or in sour milk curds. To a large extent the control of these flies depe on cleanliness, i.e. removal of the attractive fermenting or decaying mat It is probable that the D.D.T. spraying mentioned above will be effective aga these flies too.

Mosquitoes. It is extremely improbable that any mosquito would bree an animal house in Britain, but in certain country districts they might com in numbers sufficient to cause annoyance. Even in temperate countries t might bring in infections, e.g. tularæmia in Sweden, equine encephaliti North America. The control of mosquitoes, by dealing with the water which they breed, is a large complex subject outside the scope of this b Their exclusion by wire screening would sometimes be cheaper and n satisfactory.

7. Cockroaches

Trouble from cockroaches is particularly likely to occur in heated ani houses ; and the higher the temperature the faster will they breed. There three kinds which may be encountered : the Oriental and the American c roaches, which are large, dark-brown insects (" black beetles ") and smaller yellowish-brown German roach (" steam-fly "), which is about when fully grown. Their Latin names are, respectively, *Blatta orient Periplaneta americana* and *Blatella germanica*. The German cockroac particularly troublesome because of its rapid development and because o remarkable climbing powers.

Broadly speaking, all three species have rather similar habits and biol They hide in crevices during the day, especially near hot-water pipes. night they emerge to forage for food. Their depredations cannot be sai

very extensive ; but they soil food with their excrement ; they have a ıracteristic disgusting smell and in their wanderings they may contaminate d with bacteria. Obviously it is essential to exclude them from rooms ere infected animals are kept. In addition, cockroaches are the intermediate st of the nematode *Gigantorhynchus moniliformis*, parasitic in rats.

Unless it is possible to fumigate the infested buildings, the eradication of kroaches calls for persistent regular treatments, because the insecticide as ıle only reaches the active stages. The eggs are laid in pod-like cases which y be deposited in inaccessible places and these hatch out young roaches after) or three months. (The German roach, however, carries the egg-case ıut with it until one or two days before hatching.)

Generally the most feasible method of controlling cockroaches is by a weekly ıtment with a powder insecticide. Cockroach powders act partly by tact with the insect's cuticle and partly by being swallowed when the ature cleans itself. To be effective, therefore, the dust must contaminate insect, either directly during application or indirectly by the insect running r a dusted area. If possible, the powder should be blown into and around crevices where the insects hide. It may be possible to buy a small hand ting-gun, or something may be improvised.

The most effective dust is a 50 : 50 mixture of pyrethrum powder and ium fluoride. Sodium fluoride alone is nearly as good, though not quite rapid. It must be remembered that this chemical is rather poisonous if en by man. A good, non-poisonous mixture which is economical with ethrum is the following :

Pyrethrum powder (0·5 or more per cent. pyrethrins)	10 per cent.
Boric acid	40 ,,
Kaolin (diluent)	50 ,,

the time of writing (1944) there are one or two new non-poisonous synthetic ecticides which appear to be very promising for use as cockroach powders. ough not at present generally available, they will probably be on the market rtly.

8. Insect Pests of Stored Foodstuffs

Infestation. The dry foods most commonly stored for experimental animals oats and bran, but many other substances may be kept in different cases. ese food products come from bulk supplies in warehouses which may be to ıe extent infested with certain insects. The most commonly encountered small brown grain weevils (*Calandra granaria*) and dull-grey flour moths *hestia kühniella*) ; but a number of other small beetles are often found. younger stages of most of these insects are grubs or caterpillars ; those of grain weevil may be quite hidden inside cereal grains.

On delivery, the food will probably be only lightly infested so that the insects hard to find. In such small numbers they are harmless to the animals ng the foodstuff. But if the food is kept for a long time they will breed multiply. Heavily infested food can be completely spoilt because, apart n the loss in nutritive value, the moisture produced by the insect's abolism makes the food damp and encourages moulds and mites to pro- ate. Heavy infestations of mites sometimes occur in the absence of insects od is kept for a long time under somewhat damp conditions.

Food infested with insects or mites can be sterilized by heat or fumiga as described below. But, provided the food is in good condition on deliv the trouble can be avoided if stocks of food are not kept too long. When a delivery arrives it is advisable to use up the old lot first ; if the bins are h tually filled up with the new supplies there will always be a pocket of old f and perhaps pests, at the bottom.

Sometimes food which is infested to such an extent as to be unfit for hu consumption may be supplied for animal use. It is important to sterilize s consignments as soon as possible to prevent further degeneration.

Disinfestation. Dry foods (wheat, bran, flour, rolled oats or maize, can be steamed. Moist, sugary or fatty foods (fishmeal, yeast or dried fr should be fumigated, though this is rather more troublesome.

The temperature necessary to kill insects is not high ; 10 to 15 minute 60° C. will kill any insect pest. But to allow for penetration of heat throu mass of food, 1 to 1½ hours at 100° C. may be necessary. The food shoul steamed in closed tins at atmospheric pressure, the lids being kept on du subsequent cooling to conserve moisture. Biscuit tins do very well for purpose. For a steamer, an iron box or tank with a well-fitting lid is neces and the tins are put on a shelf over a few inches of water which is kept bo at the bottom.

To fumigate foodstuffs an ordinary dustbin will serve. It can be m reasonably gas-tight by improvising a gasket from thick rubber pressure-tu fixed round the top to give continuous contact with the lid. The best wa expose the food is to construct a framework to carry separate shelves or t so that the food is exposed in layers 3 or 4 inches deep.

Carbon disulphide is an effective fumigant used at the rate of 500 c.c. cubic metre with an exposure of 24 hours followed by 24 hours' airing.

9. Rats and Mice

Rats and mice can become extremely troublesome in animal houses if are not checked. Apart from destruction and contamination of foodstuffs damage to structure, they are liable to distribute parasites among the st The original source of many infestations of laboratory animals could be tr to these pests. In addition, hungry rats are quite capable of killing s laboratory animals.

Food stored for the experimental animals is a great attraction for rats mice. It should be stored in bins, metal dustbins for preference. Also, must not be spilt on the floors. To live permanently and breed on the prem rats and mice need suitable harbourages. Rats naturally require more tection than mice and are not likely to infest buildings unless there are u quented cellars or extensive wall spaces and conduits. They may, howe visit the animal house at night from outside ; but this can be prevente barring such entries as broken ventilators and open windows. The c under a badly fitting door may be enlarged by a rat to allow ingress. That be prevented by a metal plate along the bottom of the door. Remember food refuse *outside* the house may attract rats to the neighbourhood. should be stored in a metal bin while awaiting disposal.

In sound new buildings, especially those with solid walls and concrete fl rats and even mice will find it difficult to breed. The structure of an exi

ilding can be improved to some extent by stopping mouse-holes with ment. Quite small crannies should receive attention because mice can pass rough a very small aperture. One important point is the space round pipes ssing through a wall.

In an old wooden-floored building it may be quite impossible to exclude ce entirely. The numbers must then be kept low by periodic trapping. ce are readily caught in break-back traps, the important thing being to use arge number at once. The best sort have a flat treadle on to which the mice run; these should be baited with flour or rolled oats. For the kind with e prongs instead of a treadle, a good bait is a stale crust fixed to the prongs. The traps should be set at right-angles to and against the walls and other ces where the mice are running, not out in the open.

If, in spite of protection of food and attentions to structure, there is per- ent trouble with rats, it may be necessary to plan a rat extermination npaign embracing neighbouring buildings.

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CHAPTER 4

THE RABBIT

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With a section on Diseases

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1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Handling and general management. 6. Anæsthesia and euthanasia. 7. Diseases.

1. Introduction

For several centuries it has been the practice to keep rabbits in hutches. such conditions many variations have occurred and been developed, until day there are scores of different varieties, in many colours, types and sizes 5 to 15 lb. adult live weight). This multiplicity of sizes calls for variations housing and management which sometimes appear confusing. The larger eds tend to produce larger litters and are more susceptible to dietary iciencies and to sub-normal management than are some of the smaller breeds; y are at a disadvantage also unless provided with large hutches.

In the selection of breeds suitable for laboratory work due regard must be en to the purpose for which they are required. Thus for skin tests rabbits h as the Himalayan, the "Copenhagen" (so named in Great Britain because vas obtained from Madsen of Copenhagen) and the New Zealand White ich is similar to, if not identical with, the "Copenhagen") are pre-eminently able. On the other hand, for work entailing repeated intravenous inocula- and serial bleedings large rabbits with large ears such as Lop, Half Lop, mish Giant and Belgian Hare and their crosses are the most useful. The ll hardy Dutch rabbit is widely employed for diagnostic purposes. Other eds in demand by laboratory workers are the English, Chinchilla and veren. Some valuable notes on the specific experimental uses to which erent breeds have been put are given by Herrlein (1940).

Once a laboratory has established its stock of rabbits a policy of inbreeding enerally pursued. By this means the risk of reintroducing diseases which e been eliminated is reduced and the stock becomes more uniform in regard growth-rate, resistance to disease, and response to antigenic stimuli. The ue of inbred strains is exemplified by the work of Lurie (1941) on the ctive inbreeding of strains with enhanced or diminished susceptibility to erculosis.

The mimeographed bibliography, *The Rabbit as Used in Disease Re-ch*, compiled by Carlton M. Herman of the Fish and Wildlife Service, U.S.

Department of the Interior, was no longer available when this article we press but Dr. W. B. Bell, Chief of the Division of Wildlife Service, has indic that it might be revised and reprinted if there were a sufficient demand.

2. Accommodation

Rabbits will thrive in either outdoor or indoor hutches, but they mu protected from the weather and from their natural enemies, which incl de Wooden hutches are suitable for breeding and maintenance purposes bu most experimental work, e.g. bacteriological or nutritional, all-me al which can be sterilized are desirable. Hutches should be free from drau since these raise the incidence of respiratory diseases, but should have ventilation at the front. The floors should be kept as dry as possible, wet warm cages provide a favourable atmosphere for the developmer coccidia. Hutches must be made waterproof. Roofs of outdoor hu should have a 6-in. slope, from front to back, covered with strong felt or waterproofing made to extend 4 in. to 6 in. beyond the back to shute the clear of the backs. Hutch floors should be painted with hot pitch and all to dry before being occupied. Indoor hutches are more convenient for wo with experimental animals and are raised 9 in. from the floor and arranged tiers high. Hutches must be spaced to leave room to use a wheelbarrow bet the rows of cages for feeding and cleaning, e.g. 5-ft. passages.

In view of the multiplicity of breed-sizes and crosses hutches of dimensions are used, but many of them are disgracefully small and pre the occupants from taking reasonable exercise, and from keeping away fro soiled corner. After comparative tests with many different hutch-desig was found that a *minimum of 1 sq. ft. of floor space should be allowed for 1 lb. adult* live weight. Therefore a Dutch weighing 5 lb. would then ha hutch 2 ft. deep by 2 ft. 6 in. long, or a Chinchilla of 6 lb. 2 ft. × 3 ft, Belgian of 8 lb. 2 ft. × 4 ft. However, to simplify labour n managemen hutches should be standardized, and to meet this requirement a ge purpose hutch (Fig. 20) was evolved (King Wilson, 1935). It is 2 ft. (back to front) and 2 ft. 6 in. long. Between two hutches is a door hing the ceiling so that it can be opened up when a doe has a litter, to give her d space. (For giant rabbits allow 1 ft. extra length.)

Nest boxes are removable; dimensions 14 in. × 14 in. or slightly l In cold districts and with outdoor hutches nearly half the hutch shou boarded off as a breeding compartment, with a separate wooden door.

For breeding and maintenance purposes wooden hutches, or wood asbestos or wire floors, are commonly employed. Bedding consists usua sawdust, wood-shavings, peat-moss or chaff as absorbent material, bu nest-making grass hay is best, with straw as a substitute.

Food vessels may be of glazed earthenware for simplicity in cleaning these break if they fall out of the hutch. Enamel bowls are preferable pro they cannot be readily tipped over. Water-tins can be hung on the ins the door, but should be low enough for the rabbit to see the contents. Ra will drink readily from water-bulbs (Fig. 14 (*a*)). Wire racks for hay greenstuff can be made of netting of heavy gauge with 1-in. mesh.

Convenient boxes for carrying rabbits are made of plywood, meas approximately 14 in. × 10 in. and 12 in. high for each rabbit. Venti

ould be in the top of the sides or in the lid, not at floor-level, and the boxes ould be waterproof. Two- or three-compartment boxes are convenient.

For experimental purposes Dewar (1945) keeps rabbits separately either in l-metal-sided huts or in simple all-wire cages. The large metal hut illustrated Fig. 22 is a modification of the pattern in use at the State Serum Institute at penhagen. Its dimensions are: length 23 in., width 16½ in. and height ½ in. The floor, back and two sides are of sheet metal; the lid and centre

FIG. 21.—GENERAL-PURPOSE HUTCH DESIGNED BY KING WILSON.

nel of the front are of 1-in. wire caging. The lid is removable and the front ged at the bottom to facilitate cleaning. The centre wire panel is hinges to permit the feeding and removal of the rabbit. Wood chips are used ce ring. The all-wire cages (length 19½ in., width 14½ in., height 11½ in.) ed with a hinged front and a ½-in.-mesh wire floor grid. The cage etal tray 2 in. deep and containing sawdust, the floor grid being d. Details he sawdust. The litter in the trays is changed twice weekly um torized by

is replaced by a clean one each week. Dirty cages are autoclaved and wa
before further use. The metal-sided cage is preferable to the all-wire ty
it is roomier and cleaner. The wire type, however, is much lighter and
cumbersome and is preferable when large numbers of rabbits have to be m
about frequently.

Single infected rabbits are kept in cages of the dustbin type describe
Miles & Mitchell (1934).

[Blount (1945) provides an illustrated account of the successful use o
" Milanson Hygienic Hutch Unit " which consists of a solid platform w
small hole through which droppings and urine fall to a tray below. The r
is at first coaxed to the platform by sprinkling the latter with a small qua
of soiled bedding. Apparently it is not difficult to train rabbits to use the
and Blount records that in the cases in which he tried it all the urine and a
80 to 85 per cent. of the fæcal pellets were collected in the trays.—*Ed.*]

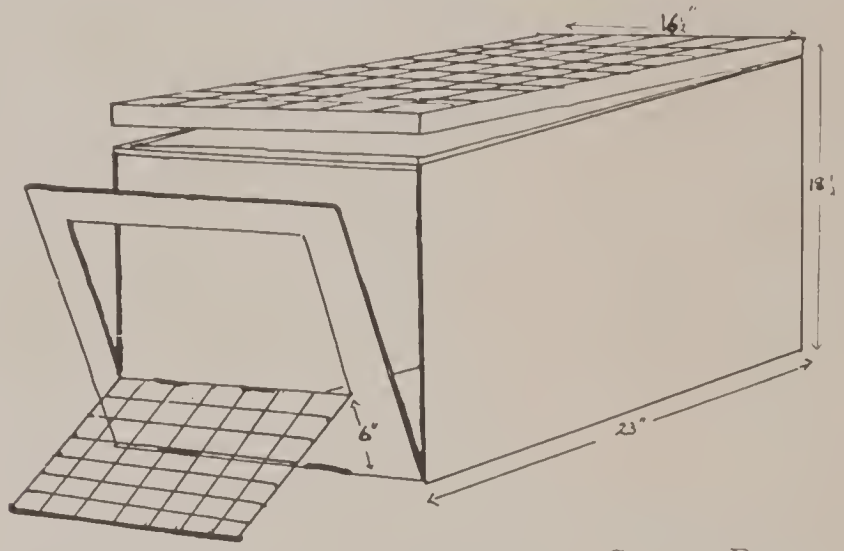

FIG. 22.—COPENHAGEN TYPE CAGE FOR RABBITS OR GUINEA-PIGS, AS USED BY DEWAR.

3. Nutrition

Nutritional requirements. In the wild state (Southern, 1940*b*) the r
is herbivorous, but under domestication many changes have been bro
about, notably an increase in size. Breeding practices vary greatly acco
to available foodstuffs and the composition of the diet is very variable fro
season to another. The domesticated animal requires supplements of
centrated foods in addition to greenstuff and hay for consistently succ
production. There is a dearth of knowledge of the precise nutritional
of this species. Synthetic diets have been used by different workers (Ma
et al., 1935) but none of them has been entirely satisfactory over prolo
periods, although more encouraging results have been obtained with add
of yeast and combinations of plant extracts (Hogan *et al.*, 1934, 1936, I
Further work is needed to devise synthetic diets satisfactory for succ
the production.

Various estimates of the nutritional needs of the rabbit have been
(Sherwin, 1933 ; Hagelin, 1935 ; Tomme & Loria, 1935 ; Templeton, Ashl
greenstuff, 1942 ; Stevenson, 1943) but additional data are needed b

Convenient standards are generally accepted. (For further discussion
approximately, McCartney, 1940.)

The great length of the small intestine of the rabbit was at one time believed e a provision for dealing with bulky foodstuffs and for enabling it to utilize , but it has in fact been shown that this fraction of the diet is poorly utilized omparison with ruminants (Watson & Godden, 1935 ; Watson & Horton, 5 ; Raymond, 1936) in spite of the practice of coprophagy. So far as ein utilization is concerned the mere quantitative measurement of the unt present is not a reliable guide to its nutritive value, since digestibility nds upon the quality of the proteins (Crampton, 1934).

Not only the protein metabolism but also that of cellulose, various vitamins minerals are influenced by the practice of coprophagy or pseudo-rumination rot, 1882 ; Madsen, 1939 ; Taylor, 1939 ; Eden, 1940 ; Blount, 1945, 17–20) which is also observed in wild rabbits (Southern, 1940 *a*, *b*). The bit passes two types of fæces, the " day " fæces which are those commonly served in the hutch or on the ground, and the " night " fæces which are rmally taken and swallowed directly from the anus. Eden has shown that e two types vary considerably in composition. There is every reason to pose (Kon, 1945) that coprophagy provides the rabbit with abundant plies of various B vitamins formed by microbial synthesis in the cæcum and ge intestine and later absorbed from the upper parts of the intestinal tract. e complicating effect of coprophagy upon the absorption of copper has been wn in a later paper by Eden. For studies on the microbial population of the cum of the rabbit, see Baker & Martin (1937), Baker (1944).

The vitamin requirements other than those for the B complex, which in any nt is usually richly supplied in the normal dietary, have not been adequately ermined. Vitamins A and D are both essential, and may be deficient in tain practical rations. The requirements of rex rabbits appear to be higher n those of other breeds (Nachtsheim, 1930 ; King Wilson, 1934). Vitamin deficiency leads to keratinization of the mucous membranes, keratomalacia l xerophthalmia (King Wilson & McCartney, 1940), as well as to bony rgrowths (Mellanby, 1944). Vitamin D is essential for proper calcification he bones and teeth. Both vitamins may be supplied by the addition to the ion (for does and young) of 1 per cent. of cod-liver oil or its equivalent, but e should be taken not to produce cod-liver-oil injury which now appears to identical with nutritional muscular dystrophy (Harris-Stewart, 1929 ; es, 1929–30, 1931 ; Harris & Irnes, 1931 ; Turner, Meigs & Converse, 6 ; Morgulis & Spencer, 1936).

The cod-liver oil in excess causes oxidative destruction of the vitamin E. tection may be obtained either by feeding the fish-liver oil on certain days y or by administering it on a cabbage leaf, or with bran alone, before the in feed. Apart from such induced cases it is unlikely that vitamin E ciency will occur in normal circumstances, as this vitamin is present in siderable quantities in the usual types of ration. Although experimental ciency of vitamin C has been reported (Mellanby & Killick, 1926 ; Mellanby, 0) there is so far no evidence that the rabbit does not normally synthesize its n requirements. (A paper on vitamin C requirements is provided by Bruce Parkes, see p. 95.)

The mineral requirements of the rabbit have not been well studied. Details ting to calcium, iron, copper, iodine, sulphur and salt are summarized by

King Wilson & McCartney (1940). Kale and certain other green foods clover are well supplied with minerals, but some green foods and root deficient. In North America salt is commonly added at a level of from 0· 0·5 per cent. of the total ration (Butterfield & Lloyd, 1927) or as 1·0 per of the mash. Salt-consumption is higher for breeding does than for l (Templeton, 1938) and in general the American practice may be followed, salt brick provided. The salt tolerance of the rabbit is high (Gompel, Ha & Mayer, 1936 *a*, *b*).

Water requirements. More than two-thirds the weight of the new- rabbit and approximately two-thirds of the adult is water (King Wilso Morris, 1932). The water requirements of the rabbit are high, especial hot weather; rabbits kept in the sun lose from 12 to 23 g. of water per ho compared to 2 to 3 g. per hour in the shade (Hill, 1920). Resting adults consume a quarter of a pint daily and this figure may rise rapidly to high l in hot, dry weather and during lactation. The milk yield of does may be as as 180 g. daily and maintenance of milk yield is impossible without a c sponding supply of water. Water-deficiency retards growth and develop (Wirth, 1933; Christian, 1935–36). Even with mixed rations conta succulents water should be given. Kennaway (1943) has provided evid that the moist-food system adopted as a substitute for giving laboratory ra fresh water is unsound and should be considered obsolete. He has stresse fact that widespread ignorance exists on a question which is urgent fr humanitarian point of view. Although the drinking habits of indiv rabbits vary (Bruggemann, 1937), water should always be provided in dri which cannot be overturned, or in bulbs (Figs. 14, 15). Certain vicious h such as urine-drinking or cannibalism may result from the withholdin water, and may be cured by the provision of a daily supply of clean w (King Wilson, 1943).

Feeding-stuffs. Rabbit rations consist usually of a mixture of green or roots, hay, and concentrates such as cereal grains or mash, fed in two n daily. An additional feed can be given to young stock at mid-day.

Green foods vary greatly in their feeding value but broadly speaking kale the cabbage crops are the least variable. Scythed grass decreases in nutr value as it becomes old and stemmy, particularly under drought condit and as its fibre content rises so its digestibility declines. Excessive quant of woody fibre may adversely affect the digestibility of other constituen the ration. Leguminous plants such as clover, lucerne and sanfoin are gene more suitable than the coarser kinds of older grass. The writer has fo that rhubarb leaves are a satisfactory alternative to grass (King Wi 1946).

Many weeds are used for feeding, e.g. hedge parsley, dandelion, shepl purse, sowthistle, plantain, groundsel, vetches, coltsfoot and chicory, but collection is a slow process unless they can be mown. It is desirable, there to have green crops cultivated for the requirements of the rabbitry, for o wise there is a grave risk of summer green foods being exhausted and ca the collapse of experimental work. A calender of succulent foodstuffs ca adapted to meet local conditions of soil and climate (King Wilson, 1 For the system in use at the Institute of Animal Pathology, Cambridge Chapternate. 101.

Green foods contain 80 to 85 per cent. of moisture, and if fed too liberally
se excessive urination and make the cages odoriforous. Owing to their low
egy-content in relation to their bulk, green foods if fed without supplements
oncentrates to lactating and growing stock cause the animals to become
bellied and thin, with resultant high mortality (Page, 1942).

Roots and their tops, including carrot, swede, sugar-beet and kohl rabi, are
extensively for rabbits. Sugar-beet tops should be wilted at least a week
re feeding, for if extensively fed whilst fresh they are harmful owing to their
content of oxalic acid. Mangold tops are not used ; their roots are clamped
not fed until the New Year. If yellow shoots grow on roots during storage
y should be removed before feeding as they are potentially dangerous,
ecially for young stock.

Roots are fed mainly in autumn and winter, when fresh greenstuff is scarce,
compared with leafy green foods they are deficient in growth-forming
stances. This may explain in part the poorer growth-rate of autumn-born
ers. On the other hand, roots contain little fibre and compare more closely
h cereals in their dry matter content and are suitable to use for fattening.

Tubers. Potatoes contain approximately twice as much dry matter as
rots, swedes and mangolds, and are rich in starch. On the Continent, where
bit-keeping is widespread, the small chats and peelings have constituted the
in concentrated food for the peasant's rabbits for many decades. Experi-
nts have shown that 4 oz. of cooked (steamed) potatoes are a satisfactory
stitute for 1 oz. oats in rabbit rations (Page, 1942). A few raw potato
lings are often fed to fattening stock, but raw potatoes are injurious if fed
ntinuously (King Wilson, 1945), especially to young stock and to pregnant
s. Cooked potatoes have become one of the main substitutes for cereal
ins in war-time rabbit rations, and so long as they are fed in adequate
antities they keep stock in good condition. Artichoke tubers on the other
nd have proved inadequate for growing young stock (Hutchinson, 1943).

Hay. Although hay is made by drying green crops it should not be used
iscriminately as a substitute for the fresh material, since it has been shown
t this practice may result eventually in paralysis and breeding failures
ief, 1939 ; Nordfeldt, 1942 ; Vail, 1944). Hay is fed usually in addition
succulents, clover, lucerne and sainfoin hay being highly esteemed. Seeds
and meadow hay are used also, but pure grass hay is usually of low digesti-
ty. Dried stinging-nettles and air-dried lawn mowings have proved to
superior to rough grass hay (King Wilson, 1941).

Cereal grains. Oats were the most popular peace-time grain among
glish rabbit-keepers, although in digestibility they are inferior to barley,
eat or rye (Kneirien, 1898 ; Bruggemann, 1937 ; Voris *et al.*, 1940). Grain
xtures are suitable maintenance supplements, but for breeding stock and
ners should include additional protein, e.g. soya bean, linseed or similar
ls, together with a salt supplement.

Mash. Numerous mash mixtures have been used, including the grain
xture after grinding. Another mixture is four parts of cereal meals to one
lairy cake. A popular mash for breeding does with young consists of equal
ts of bran, weatings, flaked maize or barley meal, and fish meal. These
a few only of numerous mixtures which have been used (King Wilson &
Cartney, 1940).

Bran is commonly used in mashes and is the only rationed foo for rabbits

in war-time, but when fed as the sole concentrate it resulted in reduced growth and loss of body-weight on maintenance rations, by comparison with either oats or kitchen waste (King Wilson, 1943). For production there 51 per cent. mortality among the young at 10½ weeks of age, whereas in another group receiving 33·3 per cent. of bran plus kitchen waste the mortality only 2·3 per cent.

Practical rations. Numerous combinations of different foods have been fed. The following specimen diets are merely intended as a general guide not as a rigid routine.

Ration for resting adult.

Green food (grass, clover, weeds, etc., or a mixture) and/or roots.	12 to 16 oz.
Hay (clover, seeds or meadow hay)	2 to 3 oz.
Concentrates (cereal grain or meal mash)	2 oz.
or cooked potatoes	8 oz.

Owing to the great variation in live-weight and growth-rate of the many types of rabbit the quantities of food should be adjusted to appetite. Abse of prenatal concentrates results in lower birth-weight of the young (Ash 1944). When the fodder is of high-grade quality the amount of concentr can be reduced.

Ration for doe and litter.

Green food (kale or other brassica, clover or mixture)	*ad lib.*
Hay (preferably leguminous, of green colour) ..	2 to 4 oz.
Concentrates (cereal grain or meal mash plus protein)	4 to 6 oz.
Common salt	0·25 to 1 per c

In the absence of legumes and green food, steamed bone-flour should added. For winter litters, cod-liver oil is given. Weaners continue on production foods until approximately 3 months of age. After this stage concentrates may be reduced as the fodder is increased.

Caution should be exercised to avoid feeding frozen, poisonous, soi decayed or contaminated foods, or diseased and mouldy material. Fermen greenstuff should be avoided. Food should not be cooked in galvan buckets in view of the danger of zinc poisoning (Ormrod, 1943). Any chan should be made gradually. If an animal has diarrhœa, the succulents and m should be omitted and dry bran, hay and water given until it has returne normal.

Further details of rabbit feeding appear in the Imperial Bureau of Ani Nutrition's Technical Communication No. 12 (King Wilson & McCart 1940). For details of the sugar-beet method of feeding rabbits and guinea-p as practised for many years at the Department of Pathology, Cambri see Mitchell & Pleasance (1927). Feeding by means of a hopper is advoc by Mason (1944).

For a discussion of pellet and cube diets, which will probably come to employed for rabbits as well as for guinea-pigs and for murine rodents, Chap. 5, p. 102, and Chap. 6, p. 120, and also the very recent paper by Br & Parkes no'ed on p. 95.

4. Breeding

Rabbits are generally ready for breeding when they are approximately months old, but vary a month or two according to their size and environ- t. Rabbits can be bred from at all seasons of the year, although normally ng the late autumn and early winter there is a period of quiescence. There o clearly defined œstrus cycle (Hammond & Marshall, 1925). Ovulation s not occur unless the doe is jumped by another rabbit of either sex, and it takes place about 10 hours after the orgasm. The doe is taken to the 's hutch and copulation usually occurs within a minute or two, after which is returned to her own hutch. [According to Polikarteva, E. F. (1945) d in *Anim. Breed. Abstr.* **14.** 165, ovulation is due to nervous stimulation sed by the behaviour of the male and not to mechanical stimulation caused coitus.—*Ed.*] Pregnancy may be diagnosed at 10 days by abdominal pation (Paterson, 1945) and confirmed with certainty after the 24th day mmond, 1936). The gestation period is 31 to 32 days and may vary 120 hours, but if the deviation is greater than this the young are usually d at birth. Pseudo-pregnancy is not uncommon, and about 16 to 20 s after mating the doe makes a nest. If the does are mated to a buck on 18th day, however, the maximum percentage of infertile does can be detected remedied (Templeton, 1940). The doe may desert her young if she is turbed or suffers from dietary deficiencies, or as a result of close continuous reeding (Pease, 1928). Surplus young can be fostered when 1 to 2 days of . The new-born are helpless and dependent upon the doe for nearly 3 weeks, er which time they run about and supplement their diet from the doe's ion. Lactation generally lasts 6 or 8 weeks. Weaning is generally carried t from 6 to 8 weeks and the sexes are separated. Sexing at birth (Temple- , 1940) or at 3 days old is intricate but by 3 months is quite simple.

Unless the sexes are separated when $3\frac{1}{2}$ to 4 months old, fighting and wanted matings may occur. Before the litter is broken up the young stock uld be tattooed in one ear (King Wilson, 1937) for identification. In this y a complete record of the entire stock can be kept.

The most critical age for the young rabbit is at birth, but there is a second ical stage at from 1 to 3 months old unless it has been provided with an equate allowance of concentrated foods. On a poor diet it becomes highly ceptible to coccidiosis and debility during this period of maximum growth. is especially important, therefore, to provide good nutrition (a diet low in e, high in proteins and containing adequate minerals and vitamins), space exercise, dry hutches and good hygiene. Breeding animals should be cted from virile stock as some strains are more resistant to disease than ers when exposed to infection (Greene, 1935 ; Lurie, 1941).

[*Diagnosis of male fertility.* Walton (1946) writes that in the rabbit the thod of diagnosing male fertility is as follows. A glass slide is applied to the rted vagina immediately after copulation and the expressed drop of fluid examined under the microscope for the presence of spermatozoa. Many ive cells will indicate a high probability of fertility. One negative result is sufficient to prove sterility and the test may have to be repeated several es before conclusive evidence is obtained. Greater certainty can be obtained using an artificial vagina and the technique of collecting semen described by cirone & Walton (1938).—*Ed.*]

5. Handling and General Management

Rabbits should never be lifted by the ears alone. The following is a pop
method of handling them. With the left hand the rabbit's ears are grip
firmly but gently, and the right hand is then placed under its rump to take
weight (King Wilson, 1944). Alternatively the right hand may be opened
placed flat under the rabbit's stomach, but this needs more care and is a so
what risky method for handling pregnant does. Another method is to lift
animal by the loose skin over the shoulders: since the ears are frequently
for experimental purposes, pain to these sensitive parts may be avoided by
of this method (Robarts, 1944).

The method of restraint during bleeding is illustrated by Wilson S
(1931). Bleeding-boxes are not usually favoured, but it has been sugge
that if employed they should be open at the top and have a hole at the sid
the ear to go through. This permits the attendant to hold the rabbit. T
shut in by a hinged lid with the head through a hole may cause fright. [Th
(1944) has described a box for holding rabbits for ear bleeding and c
minor operations. It is claimed that this box, which is made entire
galvanized sheet iron and comprises two main interchangeable parts, is cl
durable, easily cleaned and sterilized, possesses good ventilation and re
injury to the rabbit almost impossible.] When being bled or inocu
rabbits (and other animals) should not be placed on a slippery surface.
of a secure foothold frightens and may terrify an animal. An ordinary du
sack or mat is usually sufficient for rabbits to stand on in order that they
have confidence in their surroundings.

Any animal that has been bled should be given the opportunity of drin
water. When large volumes have been taken the intraperitoneal injecti
sterile saline in volume equal to that of the blood taken would appear hum
especially in the case of rabbits which are unaccustomed to being provided
water. The addition of a little alcohol to their water encourages rabbi
drink.

If in the course of experiment it is essential to withhold food the an
should be placed in seclusion undisturbed by the distribution of food to
animals.

More attention should be paid to the avoidable discomfort of the ra
with unduly long toe-nails. Regular inspection of older rabbits and trim
of the tips of over-long nails prevents some cases of lameness and the cate
of long claws in the bars of the cage; $\frac{1}{4}$ in. of nail should be left beyond the q

Any rabbit shaking its head or scratching its ears should be examined
if ear mange be present this should be treated without delay.

Much of the information in this section has been provided by Ro
(1944). For handling under experimental conditions and management r
ence should be made to Wilson Smith (1931) and to original publications i
Journal of Laboratory and Clinical Medicine and elsewhere.

6. Anæsthesia and Euthanasia

Anæsthesia. Shackle (1944) states that "Where elaborate procedure
necessary, more especially laparotomy requiring adequate relaxation
inhalation anæsthetic is usually necessary, as the dosage of intrave

sthetics is too uncertain to secure the necessary depth without risk of piratory failure. In our hands, the administration during anæsthesia of ther doses of intravenous anæsthetic has been attended with a high mortality, the use of intravenous anæsthetics has therefore been restricted to induc-, the supplementary anæsthetic usually being ether. Trichlorethylene has en used also, but with no appreciable advantage.

" The anæsthetics most commonly used for induction are : (i) nembutal sodium iso-amytal) ; (ii) evipan sodium (hexobarbitone) ; (iii) urethane ; sodium barbitone. Of these, the first given intravenously is probably the for comparatively short procedures such as laparotomy. Like most such sthetics, the effect is somewhat variable on different animals, and a cast- dosage cannot be laid down. The usual dosage for intravenous use is en by the makers as 1 ml. (of a solution containing 6·5 per cent. or 1 *grain* ml.) for every 5 lb. of weight. While this is probably a fairly reasonable imate, we have found that a few animals are unduly prostrated by this ount, a few are anæsthetized sufficiently for laparotomy without any supple- ntary ether (a peculiarity of the anæsthesia is that the animal may make -purposive movements on stimulation, even though abdominal relaxation dequate), while a few require a substantial amount of ether in addition. s, therefore, safest to give substantially less than this, and we have found that od results are obtained by using 0·6 ml. for a small rabbit and 0·75 for a large , supplementing the anæsthesia with ether in every case. It is hard to ape the feeling that batches of nembutal vary in effect ; though this often ms to take the form of delay in attaining full effect, and it is dangerous to sh the ether anæsthesia to make good the apparent deficiency.

[Attention is drawn to the methods of intravenous nembutal anæsthesia the dog and cat elaborated by Wright (1947, pp. 168–186), in which depth anæsthesia is estimated by assessment of reflex responses and the anæsthetic e is correspondingly adjusted. According to Dewar (1945) the stretch ex evoked by pulling the pad between the toes is a useful index of depth of esthesia in the rabbit.—*Ed.*]

" Evipan sodium, given similarly, is also a good anæsthetic for rabbits but ws similar variations in effect. The usual concentration is 10 per cent., 0·6 ml. of this administered to a rabbit of average size produces anæsthesia ay be sufficient alone, but may require supplementary ether. The much more transient than that of nembutal (a doubtful advantage), abbit has usually quite recovered in about an hour, while nembutal re or less anæsthetized up to about 2 hours. If ether is given in anæsthesia is more prolonged. " While the solution of nembutal kaline, that of evipan is very strongly so and it is, in practice, rather hard the syringes which are much messed up by it.

" Urethane is usually given intraperitoneally and the effect is slow. It is al to give it in the form of a 2-per-cent. solution, the dosage being usually he order of 2 grams per kilogram of body-weight (some authorities specify ./kg.). It nearly always requires to be supplemented by a certain amount ether anæsthesia, but the method is very useful for such animals as rats or nea-pigs which are not conveniently anæsthetized by an intravenous route. lium barbitone is administered similarly, usually in the form of a 10-per- t. solution. The dosage is usually 225 mg./kg. (Gaddum, J. H., 1940, *rmacology*, Oxford University Press.)

[For non-recovery experiments on the respiration and circulation, R
berg (1944) injects 1 g. of urethane per kg. of body-weight into the ear
and allows the rabbit to recover for 10 minutes. A second dose is then inj
—usually 0·6 g./kg. is required—until the corneal reflex almost disapp
This may be supplemented with small doses—0·2 g./kg.—when required.
susceptibility is variable and slow injection is essential. Respiratory failu
induction is due to rapid injection and can usually be overcome by ox
insufflation by mouth with artificial respiration. A suitable concentrati
a 20-per-cent. solution in 0·8 per cent. saline.—*Ed.*]

FIG. 23.—USE OF ILLUMINATED BULL'S-EYE LENS IN ANÆSTHETIZING A RABBI
BY INTRAVENOUS INJECTION.

[*By the courtesy of Messrs. Willen Bros.*

" The technique of intravenous anæsthesia in rabbits is fortunately
The marginal ear vein is usually used for this purpose. This is normally
tively empty ; gentle compression of the root of the ear and a few gentle
with the finger on the ear itself usually suffice to distend the vein enoug
the purpose. It is important not to overdo the compression, as obstru
of the arterial supply defeats the object of the compression. One of the wr
assistants observed that a rhythmic compression of the root of the ear foll
by complete relaxation produced the best effect. As soon as a drop of neml
has arrived in the [illegible] is of a vein, all the veins of the ear dilate widely.

er has devised a transilluminator of the ear which greatly facilitates avenous injections. This consists (Fig. 23) of a hemispherical bull's-eye in front of a pea lamp operated by a small transformer or battery and is ufactured by Messrs. Willen Bros., London. The tip of the ear is held by operator and the ear is lightly stretched over the top of the bull's-eye. his manner every vein in the ear is very clearly seen even in black hairy oits, and even the difficult central vein can be used if necessary. The sthetic, usually diluted to about 1·5 ml. with saline, is contained in a l. record syringe, armed with a small needle ; this needle can be larger n the size of the vein seems to warrant. The vein is dilated as mentioned ve and the needle is inserted into it. This is effected much more easily n might be expected, but naturally a little practice is needed. The support the ear afforded by the bull's-eye is a great assistance to the operation. may be noted that under favourable circumstances it is possible to inject uinea-pig via the ear vein by the use of this device.) Generally speaking, rabbit does not object to the procedure ; occasionally he may kick, either the moment of entry of the needle or when about three parts of the anæsthetic already in. If an assistant is holding the rabbit and the ear is held as ve described such kickings rarely give any trouble.

" If the dosage is adequate, the rabbit usually lies quietly down and becomes conscious almost as soon as the needle is out of the vein. This is especially case with evipan. One or two peculiar phenomena may be noted. Rarely rabbit gives one convulsive kick when already unconscious, and a rapid mor of the fore-legs is occasionally seen, especially with evipan. Rarely a mentary tonic spasm occurs. A most interesting phenomenon, however, ich is very commonly seen with either of these anæsthetics, is the occurrence nystagmus, with the slow component usually to the right but occasionally the left. This nystagmus may be very slight and requires close observation see it, but it is often very striking and may be associated with simultaneous l synchronous nystagmoid movements of the whiskers or even in rare ances of the whole head. On at least one occasion the rabbit has assumed typical cerebellar position, with the head curled right round on to one ulder.

" Breathing is usually quiet and regular ; with the above dosage the oils are not much enlarged, if at all. The anæsthesia appears deceptively p, and the commencement of ether anæsthesia may give rise to some such ction as coughing, breath-holding or screaming ; the depth of anæsthesia however, usually sufficient to prevent purposive movements.

" Before proceeding to consider the technique of ether administration it y be well to mention one or two further points with regard to nembutal esthesia. It is best to avoid this drug when experiments necessitating rease of respiratory rate or excursion are being carried out. In spite of fact that the normal rate of breathing does not seem to be affected by the g it definitely neutralizes the stimulating effect of such drugs as normally se an increase in rate or depth of respiration, notably such a drug as coramine kethamide). For this reason it is of little use to try to revive a rabbit with amine when failure of respiration has occurred after nembutal anæsthesia, ner from overdose of the drug or from excess of ether to supplement it.

" Such respiratory failure is occasionally seen, and tends to be rather a l occurrence. With prolonged artificial respiration, carried out as in a

new-born baby by a method like that of Eve, it is sometimes poss re-establish breathing before stoppage of the heart occurs. The rabbit by the hind legs with one hand and by the back of the neck with the oth is tilted alternately head and tail downwards, the abdominal viscera ac a piston on the diaphragm. Coramine when given at such a time ha known to 'snuff-out' a rabbit at once, presumably on account of the tra but marked fall in blood-pressure that occurs in the first quarter of a m before the prolonged rise of pressure begins.

"We have never had any success to date with phrenazol (pentamet tetrazol) in cases of apparent over-dosage with nembutal.

"Occasionally when failure of respiration occurs (or after some ar respiration has been given for such a failure) it may be found that single r tory excursions can be elicited reflexly by a gentle staccato push in th below the axilla. After a refractory period that seems to vary in lengt the depth of the anæsthesia, this can be repeated, and in this manner ar respiration can be maintained with a minimum of effort.

"It should not be thought, from the above description, that respi failure is a common occurrence; with experience of anæsthesia it should b a rarity. On the other hand, individual rabbits vary, like human bein their response to anæsthesia and a dose of anæsthetic that is quite inade for one may kill another. It is also important to be sure that all the venous anæsthetic has actually entered the vein. This is easily seen if t transilluminator is used, when it can actually be seen flowing down th and mixing with the blood. Occasionally it leaks down alongside the v the root of the ear, which feels thickened and larger than its fellow. swelling starts to rise in the vicinity of the needle point it is, of course, a cation that the needle is not properly in the vein. If ether anæsthesia i induced there is a tendency to overdo it in the attempt to obtain ade relaxation; subsequently the nembutal becomes absorbed and comes action with possibly fatal results. Care should, therefore, be exercised to e that all the anæsthetic enters the vein, and under no circumstances s any that is extravasated be made good by supplementary dosage.

"Supplementary ether anæsthesia may be given either by dropping on to a specially constructed mask or, much better, by bubbling air th it in a bottle. If a large bottle is used it may suffice to blow air over the su of the ether or over a wick soaked in it. On the whole the simplest mea are best. The sole disadvantage of a bottle is that the first whiff of ethe cause the rabbit to jerk sufficiently hard to upset it.

"For a mask we use a cylindrical tin with rounded edges about $2\frac{1}{2}$ diameter and 3 in. deep. A number of perforations are made in the bo and a layer or two of lint placed inside the tin, covering these hole held in position by a few cross-wires. A side-tube is soldered into the t the facepiece side of the lint. A rubber tube connects this to the bottle.

"We use one from a Shipway's warm ether apparatus, but a bottle w rubber cork pierced by two tubes, one reaching to the bottom and on inside the cork, is just as good. The dipping tube is connected to an insuf rubber bulb with valves (or a foot bellows), by means of which air is d through the ether. The other tube is connected to the mask. This meth convenient and economical of ether, but the ether may be applied from a

le to the lint through the perforations in the bottom of the tin if desired. ither case, the opening of the tin is placed over the rabbit's nose.

Whichever method is used, it is important to begin administration of r cautiously, as otherwise the same difficulty may be encountered as those id in human beings, struggling and prolonged breath-holding.

Once the anæsthesia is deep enough to begin the operation without x movements, the depth of anæsthesia is assessed by the respiration and degree of relaxation found in the abdominal and limb musculature occa- ally seen in human beings. The rabbit, like the human being, sometimes is to 'go to sleep' under anæsthesia until some extra stimulus, like pulling the peritoneum, 'wakes him up' (needless to say, not to consciousness) the usual unpleasant *sequelæ* of muscular contraction of the abdominal and extrusion of abdominal contents through the incision.

"When adequate relaxation is obtained, the abdominal wall can be pulled ward well clear of the contents. At this stage a careful watch must be kept the breathing. The pupil is then usually enlarged in any case, but a signal beware is an unusual prominence of the rabbit's eyeball, giving a pop-eyed pearance. This usually seems to be a danger-signal, and it is wise to go efully under the circumstances. It may be noted that the ether bottle is ually kept shielded from the sun, but even with this precaution it is advisable, ecially in the summer months, to change the ether in the bottle from time time, rejecting that remaining in the bottle, as some obscure fatalities may traceable to the oxidation products that have accumulated.

"Delayed death after anæsthesia is not common, and usually seems to ur in individuals that are emaciated or debilitated by coccidia or the like. nerally speaking the rabbits recover well from anæsthesia, and are usually ding late the same evening.

"With regard to urethane, however, it is best to use this only in animals t are not intended to recover; some of them come round all right but die r.

"With regard to the methods of restraint used during anæsthetization in bits, when the above technique is used, any appreciable restraint is rarely ded. When administration of ether is begun after the administration of nbutal or evipan, some reflex movements are not uncommon, and in rabbits h strong claws it may be advisable to wrap the rabbit up in a towel to protect anæsthetist's hands and wrists. This is especially necessary where it has ved impossible for some reason to give a preliminary intravenous anæsthetic, where extravasation of such an anæsthetic has unluckily occurred.

"With careful technique, intravenous anæsthesia followed by supplemen- inhalation is a humane procedure, and there is no evidence that any reciable discomfort or ill-effect is caused."

Harris (1944) recommends the giving of atropine sulphate, 1/50 grain, for adult rabbit, half an hour before the administration of ether. For sedative poses he has found that paraldehyde, given by stomach tube in a dosage ml./kg. body-weight, usually causes the animal to lie quietly on its side for o 2 hours. For surgical anæsthesia, Harris has employed intratracheal er with success in several hundred recovery experiments. Anæsthesia is un in a glass bin, 18-in. cube, tracheotomy is performed, and a glass cannula ch is connected to a Wolffe bottle and foot pump is inserted. The tracheal nd is easily sutured with two stitches and very rarely gives any trouble

from sepsis or tracheal stenosis. For open-ether anæsthesia, Harri administers intravenously 2·5 to 3·5 ml. per kgm. body-weight of 2-pe chloralose plus 10-per-cent. urethane mixture, well diluted with 0·9-pe saline (this is a sclerosing mixture, and must, therefore, not be injecte cutaneously or insufficiently diluted for recovery experiments). Ether administered in an open mask until the rabbit is deeply anæsthetized ether is continued for 15 to 20 minutes, and the animal remains in a s full surgical anæsthesia for 1 to 2 hours.

Euthanasia. A most humane method of destroying rabbits has to be the injection into an ear vein of 1 ml. of saturated magnesium su solution (Robarts, 1944). Death follows in a few seconds and is unaccom by any struggling, in contrast to what happens when rabbits are killed intravenous injection of ether, which is locally irritant. An overd nembutal is, of course, equally effective, though more expensive. Pr sufficient skill and force are employed, rabbits may be killed painlessly by on the back of the neck, with a stick or with the hand ; this method is be recommended to the uninitiated. Death by air embolus is not recomm

Where euthanasia is to be followed by careful histological examinatio of the finer blood vascular patterns, Harper (1944) finds that stunning administration of coal-gas or chloroform is definitely harmful, but gradually increasing concentration of ether administered to an animal open a space as may be convenient has given good results.

Kleczowski (1944) writes : " If the rabbit is first killed by a knock neck and then the throat is cut immediately, the blood can be easily co through a funnel. After the animal has been killed by a knock at the nec action of the heart goes on for some time, which is sufficient for the purp bleeding. This not only prevents the animal suffering, but it also collecting the blood convenient. If the throat is cut without any pre treatment, as is done by some people, not only the animal suffers unneces but also collecting the blood is troublesome, because the animal violently."

7. Diseases

Coccidiosis. Perard (1925), Chapman (1929), Kessel (1929), Sm (1933), Becker (1934) and Peck (1934) give useful information abou disease, which occurs in two forms, hepatic and intestinal.

Hepatic type.

Cause. Eimeria stiedae, a species of coccidium peculiar to the r which enters the blood-stream from the small intestine and attacks the colu cells of the bile ducts of the liver, in which the protozoan completes its a cycle. The sexual cycle involves the formation of the oöcyst from the z and the accumulation of these bodies in the bile ducts produces the m dilatation and thickening of the ducts which is so characteristic of h coccidiosis. The oöcysts are excreted into the gall bladder, thence reac gut and are excreted in the fæces. After an incubation period which with the conditions of temperature and humidity, the oöcysts become inf and, upon ingestion by the same or another rabbit, the life-cycle is r menced in the biliary epithelium.

Clinical symptoms. The severe form of the disease causes exte

rgement of the liver by pressure and distortion of the bile ducts and ptoms of unthriftiness and abdominal distension become apparent in ted rabbits two or three months after weaning, before which time this of coccidiosis is rarely fatal.

Diagnosis. The disease is easily detected by microscopic examination of smears of the fæces for oöcysts, but unfortunately the oöcyst count is not liable index of the severity of infestation, which can only be estimated by st-mortem examination of the bile-ducts.

Control measures. These are essentially of a preventive nature since, once blished, the disease does not reliably respond to therapeutic treatment, lough several sulphonamide preparations, notably sulphamezathine, are ned to be effective by the oral route. Prophylactic measures aim at the ination of the infective stage of the protozoan and include :

(1) The provision of sufficient floor-space per rabbit ;
(2) Timely weaning and separation of the litter from the parent doe ;
(3) Regular and frequent cleaning of the floor and sides of the hutch followed by disinfection with 10-per-cent. commercial or household ammonia ;
(4) Adequate ventilation and the use of feeding-troughs and water-dishes of the unspillable type to avoid the conditions of temperature and humidity favourable for germination of oöcysts (a fine-mesh, wire-netting floor is ideal for preventing the ingestion of droppings) ;
(5) Avoidance of feeding-stuff (hay or greenstuffs) contaminated with the droppings of wild rabbits.

[*Editorial Note.*—A mash containing 1 per cent. sulphamezathine was effective in preting deaths from hepatic coccidiosis when fed to rabbits which had received numbers of ysts sufficient to kill most untreated rabbits. Treatment was begun 24 hours after ction. At *post-mortem* examination the liver-weights of the treated rabbits were found verage 1/20th of the body-weight as compared with 1/9th in the untreated ones which died of the infection. Treatment with sulphamezathine is very effective provided it arted before the 10th day after infection (Horton-Smith, 1946). Mention might be e also of the work on disinfection with ammonia (Horton-Smith *et al*, 1940).]

estinal type.

Cause. *Eimeria perforans*, another coccidial species peculiar to the rabbit, asitizes the intestinal epithelium, causing rupture of the cells with hæmorrhage severe infestations. The life-cycle, apart from invasion of the blood-stream, imilar to that of the hepatic species.

Clinical symptoms. The severe and often rapidly fatal form of the disease cts suckling rabbits housed under conditions which are favourable to massive estation. Such rabbits exhibit a ravenous appetite, become progressively aciated, and may scour badly.

Diagnosis. The disease is detected in a similar manner to hepatic coccidi- and while an oöcyst count of the fæces is again an unreliable index of the erity of infection, the excretion of large numbers of schizonts is a more uable indication. Post-mortem examination of the intestinal wall for the ent of the lesions is the best method of diagnosis.

Control measures. Those described for the hepatic type are equally applic- e to intestinal coccidiosis.

Chemotherapy. The chemotherapeutic control of coccidiosis in rabbits now

appears to be feasible. The administration of sulphamezathine (s dimethylpyrimidine) in the drinking-water has led to striking diminu the number of deaths from coccidiosis in a rabbitry containing 30 b does. The death-rate from coccidiosis remained very high in untreated The drug was given as a saturated watery solution. This was substitu the drinking-water as soon as the doe had littered down. The treatme given throughout the suckling period and was continued for the young after weaning until they reached the age of 4 or 5 months. (J. S. Pa unpublished data 1942–1945 from the Institute of Animal Pathology, Uni of Cambridge.)

[*Editorial Note.—E. magna* is undoubtedly pathogenic and several outbr coccidiosis produced by this species have been encountered. It is a parasite of t intestine. The oöcyst is ovoidal and possesses a micropyle-feature which differ it from the oöcyst of *E. perforans* (Horton-Smith, 1946).]

Mucoid enteritis. Within recent years this disease has ranked next in of importance to coccidiosis and is probably more frequently fat apparently affects rabbits of all ages but the death-rate varies from 100 pe amongst suckling rabbits to about 33 per cent. amongst weaned rabbits. incidence of mortality amongst adult rabbits is much less, and death ge occurs in affected does just before or after littering. The only pub references are those of McKenney and the Los Angeles reports (1939– For records of the occurrence of the disease in Great Britain see Muir (Blount (1945).

Cause. The origin of this disease is unknown. Apart from the fac it does not appear to be caused by bacteria, and is very probably not a disease, its ætiology remains speculative.

Clinical symptoms. The condition is often mistaken for coccidiosis, the outstanding feature is generally marked emaciation, involving the culature of the back and hind-quarters. There is no febrile reactio affected rabbits may show a normal appetite up to the terminal stage o ness, inappetence and general weakness. *Post-mortem* examination varying evidence of digestive disturbance.

Diagnosis. The disease is easily differentiated at autopsy from eitl the types of coccidiosis by the absence of significant hepatic or inte lesions. Suckling rabbits, apart from signs of retarded growth-rate, u show no macroscopic lesion of any kind but renal erosions can be detect the majority of rabbits dying after weaning age. An occasional r surviving from an affected litter of which the greater part died at suckli weaning age, has died showing urinary incontinence and weakness of the quarters after a gradual loss of condition for several months.

Control measures. The usual precautions of disinfection and isolatio unavailing. Experimental study of the disease has shown that affected are liable to have a continued mortality amongst successive litters and survivors from such litters have a similar breeding history. From the bre aspect it is best to ensure that breeding stock is derived from rabbitries v are free from the disease.

Snuffles. The nasal discharge, characteristic of this condition, has g rise to its name. It is highly contagious, due probably to disseminati this discharge. A valuable account of it is given by Webster (1924).

Cause. Two bacterial species, *Pasteurella lepiseptica* and *Brucella bro*

ica, both members of the normal respiratory flora of the rabbit and ...rring separately or together, are believed to be the cause of this disease.
...*linical symptoms.* In the mild form the only constant symptoms are ...ated bouts of sneezing, with or without a nasal discharge, the rabbit being ...rwise normal and in good condition. The mortality associated with this ...of the disease, generally ascribed to infection with *Brucella bronchiseptica*, ...gligible, although the condition usually spreads to the entire rabbitry.
...n the chronic type of snuffles the nasal discharge becomes very marked ...gives rise to a characteristic snuffling noise. Affected rabbits progressively ...condition and die of a terminal pneumonia. The ultimate incidence of ...tality is high.
...The acute form of the disease, which is rapidly fatal, is characterized by a ...ary nasal discharge, the appearance of which is quickly followed by ...ile symptoms. Death ensues so rapidly that the affected rabbit may appear ...elatively good condition at *post-mortem* examination.
Diagnosis. The mild form is recognized by symptoms of recurrent sneezing ...n maintenance of good bodily condition and absence of deaths. The obvious ...al discharge with gradual loss of condition, resulting in a heavy death-rate, ...dicative of the chronic type. The post-mortem lesion of fibrinous pleuro-...umonia is characteristic and the causative bacterium is generally isolated ...ure culture. The febrile symptoms of the acute form with accompanying ...al discharge are unmistakable. At autopsy, acute pleuropneumonia ...or peritonitis are the outstanding findings often with a supervening ...icæmia.
Control measures. The strict eradication of infected and contact rabbits is ...most reliable method of dealing with this disease, in view of its very con-...ous properties. Intravenous injection of a soluble sulphapyridine prepara-... usually cures the acute type, but a chronic infection persists. In the same ..., intranasal insufflation with sulphapyridine powder seems merely to ...ong the course of the chronic type of infection.
Pseudotuberculosis. This disease is quite commonly encountered in the ...esticated rabbit and is essentially chronic. It is described by Will (1931) ... by Seifried (1937, pp. 31–46).
Cause. Pasteurella pseudotuberculosis rodentium, a bacterium commonly ...olved in diseases of wild rodents. The path of infection is by ingestion of ...terial soiled by the excreta of infected mice or rats or of other infected ...bits.
Clinical symptoms. The main feature of the condition is a slow progressive ... of flesh with an ultimate resultant state of emaciation.
Diagnosis. The post-mortem picture consists of primary caseous nodules ...he intestine, generally most marked in the cæcum, with secondary irregular ...eous lesions in the mesenteric lymphatic glands, the spleen and the liver. ...nt lesions have been described in very advanced cases. The causative ...*teurella* is easily differentiated from the snuffles *Pasteurella* by its motility, ...stance to bile salts, and biochemical reactions.
Control measures. The incidence of this disease is reduced by prevention of ...tamination of foodstuffs and general measures of disinfection applied par-...larly to feeding and cleaning utensils. Clinically recognizable cases should ...mmediately killed.
Tuberculosis. This is a much rarer condition in the domesticated rabbit

than is pseudotuberculosis, and usually only single isolated cases are me (Cobbet, 1913 ; Klimmer, 1923 ; Griffith, 1928 ; Harkins & Saleeby, Gray, 1932 ; Seifried, 1937, pp. 58–63.)

Cause. Bovine and avian types of *Mycobacterium tuberculosis*, muc frequently the former.

Clinical symptoms. Similar to those of pseudotuberculosis, with rapid course of infection. Lung lesions generally give rise to an in respiratory rate in the later stages of the disease.

Diagnosis The post-mortem features of the infection, whether bovine or of the avian type, are those of generalized tuberculosis, viz. n irregular necrotic areas in the lungs and liver, numerous spherical noc varying size in the spleen and kidneys, and caseous enlargement of the ass lymphatic glands. Ziehl-Neelsen's staining method demonstrates an abu of acid fast bacilli in films of the affected tissues. The intradermal tub test is not so reliable in the rabbit as in the guinea-pig.

Control measures. Immediate slaughter of infected rabbits and incin of the carcase is essential. The incidence of the disease, low as it is, m reduced by avoiding milk which may contain tubercle bacilli and foo taminated by the droppings of poultry or birds.

Necrobacillosis. This is a chronic disease which occurs sporadically a domesticated rabbits and is usually characterized by subcutaneous sw irregularly distributed over the head and body. (Gray, 1931 ; Seifried pp. 75–80.)

Cause. Fusiformis necrophorus, a filamentous bacillus, which is a n of the skin flora of the healthy rabbit and enters the blood-stream by infected skin wounds or via the alimentary tract.

Clinical symptoms. The affected rabbit may appear in good condi the early stages of the disease before the infection has progressed beyo primary subcutaneous or internal necrotic lesion. Thereafter, as the re necrosis extends beneath the skin with accumulation of greenish c material at the site of the skin wound, very commonly sustained du fight with another rabbit, the animal gradually loses flesh and its fur be ragged and dull. The insidious spread of the infection by the subcut route to other parts of the body and by the circulation to internal vital such as the liver, kidneys and lungs, where extensive necrotic areas a duced, results in ultimate emaciation and death. Infection may spr contact rabbits by the dissemination of the contents of a ruptured absce resultant contamination of skin wounds.

Diagnosis. The causal bacillus is readily demonstrated by microscop cultural methods in the caseous lesions under the skin and in the i organs.

Control measures. All skin wounds should be cleansed and dressed reliable non-irritant antiseptic immediately upon detection. Any showing skin abscesses should be disposed of as experience has proved is practically impossible to prevent by surgical and antiseptic treatme subcutaneous spread of infection from an established primary skin lesion

Rabbit syphilis. This is a relatively common venereal disease of the d ticated rabbit. (McKenny & Shillinger, 1935 ; Seifried, 1937, pp. 81–96.

Cause. Treponema cuniculi, a spirochæte resembling that which syphilis in human beings, but harmless to man and other domestic animal

Clinical symptoms. The primary lesions appear in the form of papules up pea size of a pea on denuded areas of the skin of the external genitalia. ting ulcers up to a halfpenny in size follow the rupture of these vesicles and spread to the hocks. Secondary lesions often develop on the lips, nasal tus membrane and eyelids, involving the conjunctiva and cornea. Occa- lly death follows an extension of ulceration with generalization of infection ng lesions in vital organs. Generally, however, affected adult rabbits in in moderately good condition, although young rabbits may show riftiness. Severe ulceration of the skin around the anus and genital e may cause difficulty in passing fæces and urine.

Diagnosis. The causal micro-organism can be demonstrated microscopic- in wet preparations of the exudate from the lesions by dark-ground hods and in dried films by Fontana's impregnation method.

Control measures. Incoming rabbits of both sexes should be carefully mined for evidence of the disease, especially when intended for breeding poses. Cages and hutches should be disinfected with a blowlamp after sing affected rabbits, even though the latter may have recovered from the ase. A single intramuscular injection of 0·25 to 0·35 gm. of neosalvarsan ·5 ml. of distilled water constitutes an efficient cure for the disease. Simul- eous treatment of the ulcers with 25-per-cent. calomel-lanolin ointment r cleansing with boric acid accelerates recovery.

Pyogenic infections. These are of sporadic occurrence in domesticated bits, usually involving the salivary or mammary glands or the uterus.

Cause. Staphylococci and other pus-forming bacteria. *Fusiformis necro- rus* commonly occurs as a secondary invader.

Clinical symptoms. A large, firm swelling, varying in size up to that of the , is observed between the lower jaws, or at the side if a salivary gland is olved. In the case of mastitis the teats and udder are inflamed, the mam- y excretion is purulent, and the doe refuses to suckle her young. Pyometra cognized by a chronic, purulent uterine discharge and a history of unsuccess- mating.

Diagnosis. All three conditions are characterized by evidence of a chronic l infection with suppuration.

Treatment. The abscess of the jaw, which usually shows little tendency to st, should be incised and evacuated, and the cavity should be kept irrigated y with a reliable antiseptic solution until healing by healthy granulation ue occurs. In mastitis, the pus should be removed daily from the udder by tle massage, and the overlying skin should be treated with a mild counter- tant ointment. For pyometra, frequent irrigation of the uterus and ina with 5-volumes-per-cent. hydrogen peroxide should be carried out.

Septicæmic conditions. These generally occur during an epidemic outbreak ling up to acute infections, as soon as the causal bacterium has acquired reased virulence.

Cause. Pasteurella lepiseptica and *Salmonella typhi-murium* in young rabbits *Pasteurella lepiseptica, Pasteurella pseudotuberculosis rodentium* and *non- nolytic streptococci* in adult rabbits, particularly in does following parturition.

Clinical systoms.—Elevated temperature, accelerated respiration and loss ppetite.

Diagnosis. The causal bacterium can be isolated by cultural methods from blood and internal organs.

Treatment. A course of intravenous injections of sulphapyridine-t
in a dose estimated according to body-weight has had a curative effect in
outbreaks of septicæmic infection. The usual methods of isolation of af
rabbits, disinfection of contaminated hutches and feeding and cleaning ut
and careful disposal of carcases should be practised during and aft
outbreak.

Metabolic Disorders.

Pregnancy toxæmia. This is a fairly common disease causing mor
amongst does in the terminal stages of pregnancy; for a descriptio
Greene (1937, 1938).

Cause. It is associated with endocrine imbalance arising from a distur
of functions concerned in reproductive processes.

Clinical symptoms. A few days before parturition is due the doe sh
sudden onset of dullness and inappetence. Death follows within two or
days of the appearance of symptoms.

Diagnosis. The condition is recognized by the history and the severe
changes observed in the liver at autopsy.

Treatment and control measures. These must remain undetermined
the ætiological factors have been ascertained.

External Parasitic Conditions.

Ringworm. Both types of this malady are of very rare occurrence
laboratory rabbit, mice or rats being the usual source of the infection.

Cause. Various *Microsporon* species or *Trichophyton tonsurans.*

Clinical symptoms. One type is characterized by circular, raised g
or yellowish crusts on the nose, face or ears and uncommonly on the fee
body, resulting in destruction of the hair-roots and follicles. The main fe
of the other type is the appearance of circular, hairless spots on the fur v
covering of fine, pearl-grey, shiny scales.

Diagnosis. The branching filaments and characteristic spores of
genera of fungi can be detected microscopically in wet preparations of ma
from the lesions.

Control measures. Prevention of access of rodents to the rabbit-cag
hutches. An infected rabbit should be strictly isolated if treatment is u
taken; this consists of removing the crusts with a cleansing solution and
ing with a suitable fungicide such as dilut[illegible] ncture of iodine, 10-per
salicylic acid, or a 2-per-cent. formalin salve.

Ear canker. This is the commonest form of mange in the domestic ral

Cause. Two species of mites, *Psoroptes communis* and *Chorioptes cuni*

Clinical symptoms. Brownish scabs inside the ear cause repeated scrat
at the ears with the hind claws.

Diagnosis. The mites can be recognized microscopically in wet fil
scab after heating with 10-per-cent. solution of caustic potash solu
Psoroptes communis has cup-shaped suckers on segmented stalks while *Chor*
Communis has unstalked suckers.

Control measures. The hutches in which affected rabbits have been isc
must be thoroughly disinfected. The condition is easily cleared up by soft
the scabs with a vegetable oil, removing them with forceps and applyi

Clinical mange dressing such as " Odylen " or " Folliculine " to the affected

Body mange. This is a comparatively rare type of mange in the domesti- rabbit.

Cause. Two species of mites, *Sarcoptes cuniculi* and *Notoedres cuniculi.*

Clinical symptoms. The fur falls off from areas on the face, head and roots the ears which become covered with yellowish or greyish scabs. The intense ing resulting from the infestation of the skin causes continual tearing at the ted regions with the claws.

Diagnosis. Microscopic examination of wet smears of scab preparations ted with hot 10-per-cent. solution of caustic potash serves to demonstrate mites. Both species of mites are identified by the cup-shaped suckers on gmented stalks and there is no need to distinguish between them, since diseases caused by them are similar.

Control measures. The extremely contagious nature of the condition essitates the immediate slaughter of affected rabbits and the careful disposal carcases by burning. The blowlamp is the best means of disinfecting taminated hutches. [Not all workers are agreed that animals suffering m body mange should be destroyed. Innes (1946) states that Tetmosol, nzyl benzoate and Gammexane will almost clear up this condition.—*Ed.*]

ERNAL PARASITIC CONDITIONS.

Parasitic gastritis. This condition is very rarely encountered where ade- te hygienic measures are practised.

Cause. A species of roundworm, *Graphidium strigosum.*

Clinical symptoms. Loss of condition with diarrhœa, anæmia and emacia- n where the infestation is heavy.

Diagnosis. A significantly high egg-count in the fæces is diagnostic in the ng animal. Post-mortem diagnosis is based upon an actual worm-count of contents of the stomach, together with evidence of destruction of the mucous mbrane.

Control measures. An infestation is seldom serious enough to be fatal if feeding of contaminated greenstuffs is avoided and hutches or cages are ularly and frequently cleaned. Rabbits should not be allowed to feed on ss runs where there is any possibility of contamination with animal fæces. enothiazine by mouth in a dose according to body-weight is suggested for atment of affected rabbits.

Bladder worms. This is a parasitic condition which is relatively common he domestic rabbit though very rarely lethal.

Cause. Cysticercus pisiformis, the larval stage of *Taenia pisiformis,* a tape- rm occurring in the dog ; and also *Coenurus serialis,* the larval stage of dog tapeworm, *Taenia serialis.*

Clinical symptoms. General unthriftiness in young rabbits results from ere infestation with *C. pisiformis.* Older rabbits may show abdominal ension due to a large number of fully-grown tapeworm cysts in the abdominal ity. Post-mortem examination of rabbits suffering from a severe infesta- n shows numerous fibrous tracks throughout the liver substance caused by tapeworm larvæ in the course of their migration into the peritoneum, where y settle down in the encysted state. *Coenurus serialis* cysts occur singly

in a subcutaneous position and they may reach the size of a tennis-ball host remains in good condition.

Diagnosis. The first condition is recognized at autopsy by the pres numerous healed larval tracks in the liver and of a large number of th in the abdominal cavity. The second is characterized by a large, ro soft swelling which moves relatively freely under the skin.

Control measures. The feeding of grass or other greenstuff to whic have had access should be avoided, as well as the contamination of fo or drinking-water with dog fæces. A *C. pisiformis* infestation is seldon ciently heavy to be fatal unless it follows the ingestion of grass soiled by a tapeworm segment excreted in dog fæces. *Coenurus serialis* cysts m removed surgically without harming the rabbit, provided they are enuc from the subcutaneous tissue without rupture and consequent liberation c contents. (Shackle (1944) records that the only case of residual paralysi spinal anæsthesia he has encountered in the rabbit was in an animal *Coenurus* encysted in the brain.)

[*Editorial Notes.*—(1) Since this chapter went to press a copy has been receive Professor Anatol Metelkin entitled *Infekcionhye I Invazionnye Bolezni Krolikov* (*In and Invasive Diseases of Rabbits*), by A. I. Metelkin & P. P. Saharov (Governme lishing Firm of Collective Farm and State Farm Literature "Seljhozgiz," Moscow p. 236). The work as a whole appears to form the most comprehensive t its kind yet published.

(2) Other diseases which have been reported as occurring spontaneously under l tory conditions include the following :

Listerellosis. This was first reported in epizootic form among young rabbit occasionally among guinea-pigs at the Field Laboratories, Cambridge, by Mu Webb & Swan (1926). Another case from the same centre was described by Pat (1940) in a young adult pregnant female Copenhagen. This case did not litter down proper time and 4 days later refused food. It was destroyed when severely ill 48 later, the pathological features including an enlarged and fiable liver studded with g white pin-head foci not sharply demarcated from the normal tissue, local peritonit an enlarged uterus containing dehydrated fœtuses (developmental age approxi 16 days) enveloped in a continuous circular layer of inspissated pus which could o separated from the uterine wall with difficulty. Profuse pure cultures of *Lis monocytogenes* were obtained from the uterine pus, spleen and liver and from the abdo cavity of one of the fœtuses. Heart blood cultures were sterile. Some further no the disease are given by Blount (1945).

Myxomatosis cuniculi. So far this virus disease, first reported from Montevid Sanarelli in 1898 (*Z. Bakt.* Abt. 1, **23**, 865), has been found to occur naturally o laboratories and rabbit farms in the Western Hemisphere. It appears to be highly s for rabbits of the genus *Orcytolagus*. In the original outbreak the first symptom note a severe blepharo-conjunctivitis, with marked thickening of the eyelids. The disc thickened and after a few days the lids became sealed with thick inspissated pus. followed swellings of the nose, muzzle, skin and mucous membrane of the anal and g openings and orchitis in the male. Small subcutaneous tumours, of a gelatinou vascular nature, later appeared over the body, especially on the ears. The animals be very ill, ceased to feed and died within 2 to 5 days of the development of symp Martin (1934–35) makes the important observation that in rabbits which are not ha to any great extent tumours do not appear over the body. He believes that it is the capillary lesions in the situations where a rabbit is usually seized that are responsi the occurrence of these tumours. The disease is highly fatal and the only practical c measure is to eliminate affected animals and contacts. (See also Hyde & Gardner, McKenney & Shillinger, 1935.)

Rabbit pox. (Greene, 1934–5 ; Rosahn & Hu, 1935.) This highly contagious fatal disease has been reported from stocks of rabbits in the Rockefeller Institute other laboratories in the U.S.A. In these outbreaks the incubation period was 5 to 11

ymptoms varied with the situation of the pock-like lesions but included macular and, papular eruption of the skin (especially on the ears) and mucous membranes (includ- ose of the mouth and tongue), high temperature, prostration, blepharitis, enlarge- and induration of lymph nodes and orchitis. Small nodular lesions were present the liver, spleen and lungs. In one extensive epidemic the mortality rate amongst animals was 71 per cent., being highest in newly-weaned stock, while in adults it nly 14 per cent. Amongst adults, pregnant and lactating does were the most ly affected.

ter recovery no carriers remained. Immunity lasted for several months and there vidence of the transference of passive immunity from immune doe to young.

al papillomatosis. Parsons & Kidd (1936) reported the existence of a virus disease g papillomata on the mucous membrane and usually on the under-surface of the e. The papillomata were discrete, sessile or pedunculated, filiform or fungiform pro- ns and were epitheliamotoris in structure.

oxoplasmosis has been reported and several workers believe that it is due to the same ite as is capable of infecting the rabbit, mouse and other species (see the paper of eau & Nodenot, 1936, cited in Chapter 5, p. 107).

Hydrocephalus. Hyde (1940) reported an epidemic of hydrocephalus among experi- tal rabbits, but was unable to ascertain the cause.

Thrombo-endocarditis, arterio-sclerosis and other diseases of the cardio-vascular system. re & Ravenna (1938) reported the experimental production of thrombo-endocarditis bbits by the injection of various preparations of serum and milk. They do record, ever, that the condition may occur spontaneously in a small percentage of rabbits. Spontaneous sclerosis of the aorta and other large arteries is also encountered occasion- and may mislead experimentalists. The use of the rabbit for experiments involving npts to demonstrate the transmission of infective agents associated with cardio- ular disease has not always yielded satisfactory results on account of these spontaneous itions.

terility associated with Pasteurella septica and a Brucella bronchiseptica-like organism. w (1945) has reported the association of *Pasteurella septica* and a *Brucella bronchi- ca*-like organism with sterility in strains of rabbits kept at the Animal Research Station, bridge. Breeding troubles had been encountered in these strains, affected does on *mortem* examination showing enlarged uteri filled with a cheesy sticky pus. There been a severe outbreak of snuffles in the stock some years previously. *Pasteurella ca* was recovered from eight out of the ten does selected from those which had bred ularly or become sterile and the *Brucella bronchiseptica*-like organism from the other *Pasteurella septica* was recovered in penis swabs from four of five bucks with fertility records, and both organisms from the remaining animal. Evidence was ined from transmission experiments that *Pasteurella septica* could be conveyed into uterus from the vagina by spermatozoa.

cute hæmorrhagic typhilitis. Innes (1946) reports frequently finding this *post mortem* suggests that *Clostridium welchii* may be responsible. Blount (1945) maintains 90 per cent. of all cases of diarrhœa in the rabbit can be attributed to non-parasitic rders of the cæcum, whereas disorders of the small intestine and liver, including all s of coccidiosis, do not cause diarrhœa. He says that typhilitis is most severe in half- n rabbits, although it occurs at any age and that there is a definite seasonal incidence, most cases occurring in the early months of the year and fewest in the summer ths. There is said to be rapid loss of condition and death within a few days. On *mortem* examination hæmorrhagic, anæmic and œdematous forms are described.

ppendicitis. Inflammation of the appendix is not common in the rabbit although, aker (1944) and other workers have noted, there appears to be a normal absorption cteria through the wall of this organ. Blount (1945) states that pseudo-tuberculosis e commonest cause of appendicitis in the rabbit and he describes also a second type, hich he gives the name follicular appendicitis, consisting of very pale pin-head lesions beneath the peritoneum.

mpaction of the colon. This condition is popularly termed " the blows," since excessive ormation results from the obstruction and is noted by Innes (1946) as a frequent e of death in stock rabbits. Blount (1945) states that it occurs most commonly in grown rabbits during the summer months of the year and his own cases have been iated with the feeding of clover. The *post-mortem* findings recorded by him include ened dry and firm cæcal contents, with fæcal matter adherent to the lining membrane

in severe cases, differential states of activity of parts of the intestines, excess of ga stomach in some cases, and congestion of the lungs attributed to mechanical pres

There would appear to be analogies between this condition and certain disc cattle associated with clover feeding.

Subcutaneous abscesses. Blount (1945) considers that where large numbers o are kept about 2 per cent. may be expected to die, or to have to be killed, as a local injuries with consequent abscess formation. These injuries he attributes m fighting and in fatal cases he records that the liver is often degenerate, brown a " feathery " markings. He advises early separation of the sexes, separation o which have been in service from unmated bucks, the maintenance of small units avoidance of wire mesh in breeding compartments.

Torticollis. Innes (1946) records this as a frequent *post-mortem* record in stock Blount (1945) provides an illustration of a case but does not appear to regard common condition.

Spontaneous paralysis of the hindquarters. Jabotinsky (1936), according abstract cited below, recorded the histological findings in the tissues of three case had shown spontaneous paralysis of the hindquarters and for which no satisfactory e tion had been offered. Details were given of the multiple necrotic foci scattered t out the grey and white matter of the lumbar section of the spinal cord. Similar were seen in the nerve ganglia. No conclusions were reached concerning ætiology.

Injuries such as broken back and broken leg are not uncommon among lab rabbits and may escape accurate diagnosis. A careful examination for such should always be made. Blount (1945) reports the occurrence of broken back and attributes it to the fact that the accident is due to a sudden leaping up fro while the animal is sitting partly in and partly out of the sleeping compartme consequent rupture of the lumbar region.]

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CHAPTER 5

THE GUINEA-PIG

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1. Introduction. 2. Stock. 3. Accommodation and equipme
4. Diet. 5. Handling and marking. 6. Anæsthesia and eut
nasia. 7. Disease and its control.

1. Introduction

The principles involved in the keeping and rearing of guinea-p
experimentation are the same as in the husbandry of all other lab
animals. The aim must always be to promote the comfort and well-b
the animals and to produce guinea-pigs free from such defects as will inv
the results of experiments in which they are used. Such defects are
caused by diseases, either of infectious or parasitic nature, or by defi
due to inadequate diets. Furthermore it is desirable to raise, so far as p
standard guinea-pigs. The variation in size and weight between ani
any given age should not be great, and there should not be great diff
between guinea-pigs in their reaction to any given experimental st
The methods employed will, to some extent, depend on the type of exper
work for which the guinea-pigs are to be used, on the numbers needed
the accommodation available.

Undoubtedly the best method is for a laboratory to undertake the b
of its own animals, and to have them under its control throughout th
The following general description of methods applies to laboratories
undertake their own breeding. Differences in procedure for labor
unable to keep their guinea-pig stocks self-contained are indicated
necessary.

An essential condition for success is to have the production of a
under the control of a person skilled in the care of animals, who has a kno
of disease, and who is aware of the possible consequences of any laxity
and attention. There is much to be said for the system adopted by
laboratories of drafting junior laboratory technicians into the animal h
work there for a certain period. An alternative arrangement is to p
the more intelligent animal attendants to laboratory work. Both s
cater for the view that the care of laboratory animals is an essential
the training of the laboratory technician. However, providing the pe
charge exerts strict supervision over the subordinate staff, such staff n
be experienced. In fact, boys and girls fresh from school are suitable.
further notes on assistants, see Chapter 2.—*Ed.*]

2. Stock

Foundation stock. The guinea-pigs should be obtained from a
regarded as reliable, preferably from another laboratory, where a hist

animals and the stock they came from should be available. The guinea-selected should be inspected and weighed daily. Any unthrifty or lthy animals should be rejected, as also young animals which fail to make y gains in weight. Progeny records should be kept and only guinea-pigs satisfactory breeding performances should continue to be used. Such a ct is described by Dunkin, Hartley, Lewis-Faning & Russell (1930) with uinea-pig stock at the National Institute of Medical Research.

urchased stock. In laboratories dependent on purchased supplies, a-pigs should be obtained directly from a breeder or from a source which s any infection introduced to be traced. The animals on arrival should aced in freshly cleaned isolation quarters and kept under observation for ast a week before use. If guinea-pigs are to be introduced into a previously ontained unit, they should be kept in isolation for two months. Any ns or illness in the new stock should be investigated immediately. When ause of death is of an infectious nature, it is advisable to slaughter the n or return them to the vendor if the contract allows. If the animals are e kept, quarantine should be continued for a week after the last death. hased stock should at no time be mixed with the laboratory-bred stock. Booth (1944) points out that some breeders mix the sexes when supplying oratory so that on a long journey some of the sows may become pregnant. e should not be used for inoculation purposes or for the production of plement.—*Ed.*]

reeding. The sexes may be mixed in the proportion of one male to females. The gestation period is between 66 and 72 days. The litter be left with the mother for 12 to 14 days, or, alternatively, each young a-pig may be removed from its mother when it reaches the weight of

he female or sow may be placed with a male between a week and a fort- after her litter has been weaned. However, it is preferable to return to the breeding pens when they are in good condition rather than at an ary time. A sow has a useful breeding life of up to 2 years, and is first at about 4 to 6 months of age. Males or boars are used for one breeding n, after which they are replaced. Such discarded males are suitable for for certain types of experimental work. Under ordinary conditions, litters a year may be obtained from each sow. The average number of a-pigs in a litter varies with the stock used, but in certain colonies an ge of 3·5 has been recorded. Young guinea-pigs should be ready for ne issue at about one month of age when they weigh about 200 g.

ales and females, in the right proportion, are allowed to run together in on the floor and the females are removed when advanced in pregnancy laced in hutches. Parturition takes place in these hutches, and mother oung remain in them until the litter is weaned. However, this method essential; parturition takes place quite normally if the pregnant sows pt in pens together, after having been separated from the boars. If for reason a mother dies, her young are frequently fostered by other lactating The use of hutches is to be preferred if space is available. Occasionally re found that have acquired the habit of suckling other sows and these ls should be removed immediately.

e diagram in Fig. 24 illustrates how the breeding stock may be kept in being divided once a week into the following classes: boars and sows,

pregnant sows, sows with litters up to a week old, sows with litters u weeks old, weaners and resting sows.

[*Diagnosis of male fertility.* Walton (1946) writes that in guinea-p and mice the vaginal contents of the female with which the male ha can be removed for microscopic examination with a small spatula (a wire hammered flat at the tip and smoothed with emery paper makes tool). After fertile matings, spermatozoa can usually be seen in the and the presence of the vaginal plug will indicate that mating has tak even if the latter has not been observed. It should be borne in m live spermatozoa may be found in the vagina for only a few hou copulation.—*Ed.*]

3. Accommodation and Equipment

Guinea-pigs kept for laboratory purposes require three separate accommodation—for the breeding stock, for the stock ready for issue, the animals under experimentation. From the point of view of disease

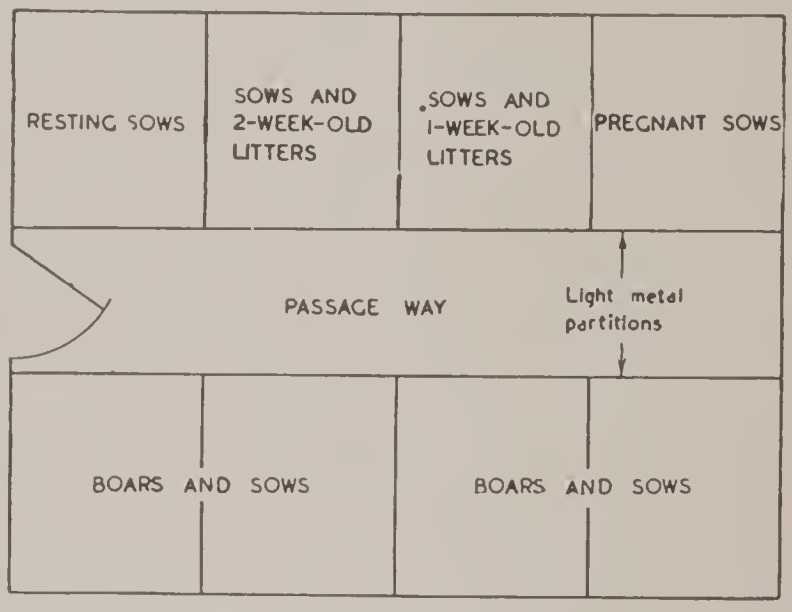

Fig. 24.—Pens for Guinea-Pig Breeding Stocks at Compton.

there is much to be said for breaking up the stock into sub-units, prefe different rooms. Outbreaks of infectious disease can be more easily in their extent and, if a policy of slaughter is necessary, the number of that have to be killed can be reduced in number. On the other han subdivision adds to the daily labour of cleaning and feeding. Howev essential that guinea-pigs under experiment be isolated from the healthy It is preferable for such animals to be housed in a different building v own sterilizing equipment and feeding utensils and to have their own att Although such optimum conditions will not always be possible in small tories, the principle of having an attendant who handles infected anima should be adhered to.

The healthy stock at these laboratories, which were designed by t Maj. G. W. Dunkin, are kept in pens 8 ft. × 6 ft. on the floor, and th 8 pens to a room (Fig. 24). About 48 adult guinea-pigs may be kept i

o 150 young growing stock. Their numbers will depend on their size. The re walled in by light metal divisions 15 in. high, which are held in upright These divisions can be lifted and the floor of the room cleaned in one tion. The floor is of hard asphalt and the floor and wall angles are rounded ilitate cleaning. The walls should be tiled or covered with water-resistant to breast height. There should be an ample supply of running water in oom. Gutters need to be well covered as they easily become choked with ng and forage. All electric wiring and piping, etc., should be covered, as end to harbour dust and dirt.

ndoubtedly heating and ventilation in many animal houses are defective. forms of heating now employed fail to keep the temperature even through- ie 24 hours. Appreciable falls in temperature, particularly in the early of morning, can and do result in an increased death-rate in the next few

uinea-pigs thrive best at a temperature of about 65° F. with humidity of between 45 and 55 per cent. It is important to keep temperature and dity relatively constant. If this can be achieved, breeding production ot be influenced unfavourably by extremes of hot and cold weather. In rection of new animal quarters for considerable numbers of animals, rovision of air-conditioned ventilation and a thermostatically-controlled ng system should be considered. However, the air-conditioning unit d create a flow of air at floor-level and the foul air should be discharged e highest point in the room. Windows should allow an ample supply of and it is best for windows to face north and south rather than east and This arrangement avoids the direct rays of the sun. Guinea-pigs, ier indoors or out of doors, should always have ample shade.

he hutches used for pregnant sows are of the two-compartment type with ll measurement of 26 in. × 12 in. × 12 in. Each compartment has a one of iron rods with $\frac{1}{2}$ in. between the rods and the other of wood, so that ompartment is dark and secluded.

ges for infected guinea-pigs are of the individual wire-and-metal type overall measurements of 14 in. × 9 in. × 8 in. and a tray $1\frac{1}{2}$ in. deep. guinea-pigs can be kept in such a cage, or up to six guinea-pigs in the larger t cage, 20 in. × 14 in. × 12 in. These cages have a $\frac{3}{4}$-in. mesh.

ges may be kept on shelves which should be of parallel metal bars, ing a free circulation of air around the cages and making the work of ing easy. Shelves may be modified by joining the horizontal bars to uprights by screw clamps. These shelves can be altered to suit cages of ent sizes and are capable of indefinite extension (Wilson Smith, 1931). stands are also made to take a definite number of cages in tiers. Such s can be mounted on wheels and moved about to suit the convenience of ttendants (Herrlein, 1940). The standard wire cages are suitable for a-pigs infected with bacterial diseases such as tuberculosis and brucellosis, hey are not suitable for animals inoculated with highly infectious material as certain viruses and the rickettsiæ. Such animals should be kept in containers 12 in. high and 14 in. in diameter, with perforated zinc nd it is desirable that the shelf uprights should stand in disinfectant

disinfectant trough should be placed so that everybody must walk gh it on entering the infected-animal quarters.

[Further information on breeding-cages and stock cages for guin
given in Chapter 2. See also cage illustrated in Fig. 22, p. 68.—*Ed.*

Feeding-utensils. In the large pens food is placed in galvanized-iro
3 ft. × 3 in. × 2½ in. For guinea-pigs in individual cages and hutche
ware containers with glazed internal surface and inturned rims are
When water is provided it is placed in upturned glass bottles with
mouthpieces working on the vacuum principle.

If a pellet diet is fed, wire-mesh racks must be provided. The m
be so designed that the pellets can be nibbled without half-eaten pell
out.

Bedding. A number of materials are satisfactory. Peat moss, p
shavings and oat husks (cavings) are all used. A bedding of 2 to 3 i
be given in the large pens. Hay also is used by the guinea-pigs a
material, particularly in the cages and hutches. Bedding should be
and protected from contamination in the same way as food material.

Cleaning and sterilization. In these laboratories cages and runs ar
out twice a week. The guinea-pigs in pens are placed in boxes, the b
removed and the whole room is swept and hosed down. The soiled
from the healthy stock is suitable for manure, but all soiled bedding
quarters of the infected guinea-pigs should be incinerated. The prov
refuse chute on the outside of the building considerably eases the re
soiled bedding from the building. If possible, guinea-pigs should be
the ground floor, but if they are kept upstairs, a lift should be inst
carrying bulky food and bedding.

Some provision must be made in all animal quarters for steriliz
equipment such as cages and utensils. Feeding vessels and cages sh
regularly sterilized. Sterilization is usually effected by standing equip
disinfectant solutions, or by the application of flowing steam. A me
tank with a metal lid is used as a sterilizer at these laboratories. Its
dimensions are 3 ft. 5 in. wide, 2 ft. 5 in. long, and 2 ft. 3 in. deep, and
obtained by gas heat.

All equipment is placed in this sterilizer before cleaning, so that ca
bedding, discharges, etc., are sterilized before they are handled during c
If possible, the sterilizing-room should lead into the cleaning-room, w
turn leads to the store or stacking-room. Such an arrangement avoids
between non-sterilized and sterilized equipment.

The use of disinfectants for routine cleaning is not to be encouraged
properly cleaned animal quarters there should be no undue smell. If
fectant has to be used as a deodorizer there is something wrong with the
methods employed or with the ventilation.

Guinea-pigs in grass runs. Guinea-pigs may be kept in the open p
satisfactorily all the year round once they have been acclimatized.
hutches with runs floored with close 1-in. wire-mesh are suitable. It is d
to have the hutches of fairly light construction so that they may be m
to fresh ground daily. This method has many advantages particularly
hygienic point of view. It should not be assumed that the access t
obviates the need for water, as in times of drought the grass will not
sufficient moisture. In late summer and winter when the grass has
fibre content, fresh green vegetables must be supplied.

4. Diet

e feeding of guinea-pigs in the past has been largely by rule-of-thumb ds, and it is only recently that their dietary requirements have undergone quantitative and qualitative investigations. The following diet has l satisfactory for routine purposes. A mixture of bran and dried sugar-ulp in the proportion of 2 to 1, is fed in the morning at the rate of 1 oz. per -pig. The beet pulp is soaked in water overnight. Crushed oats in of the sugar beet are also used but in our experience have not been found cessful. In the afternoon hay and green food are provided. The hay l be meadow hay of good quality. The harder types of hay and clover re are not suitable since they are not readily eaten, and injuries to the , eyes and nose are not uncommon.

imple succulent green food and wet mash is provided, drinking-water is cessary. [But see discussion in Chapter 4, p. 70.—*Ed.*]

e importance of green food for guinea-pigs has long been recognized and gh this to some extent is related to this animal's dependence on ascorbic itamin C), it appears that other factors are involved.

olley (1942) has presented evidence to show that besides the water-vitamins the guinea-pig requires at least three other dietary essentials wth and maintenance of life. Two of these factors—one is soluble and er insoluble in 50-per-cent. ethyl alcohol—were obtained from linseed-oil The third factor was supplied by feeding dried grass. Sober *et al.* also have investigated the nutritive requirements of the guinea-pig. very useful article can be summarized by saying that the guinea-pig rs to need three factors in addition to those that have been demonstrated essary for the rat and the chick. These three factors were supplied in ent amounts if the diet contained 16 per cent. of grass, 16 per cent. of and 20 ml. of winter milk. What these substances are, and their relation-o each other, remain to be determined. Both articles should be consulted se interested in more exact knowledge of guinea-pig dietary requirements. has been known for some time that inadequate amounts of green food in small amounts of complement in guinea-pig sera. [This is apparently ted to the ascorbic acid content of the diet (Kodicek & Traub, 1943).

ilure to supply green food may result in an epizootic caused by bacteria. y and Allen (1921) described such an outbreak caused by *S. enteriditis* ed by *Bact. alkaligenes faecalis* (*Brucella bronchiseptica*) pneumonia. eath-rate was reduced to normal within a week by the addition of green o the diet.

e following rotation of green foods is fed at the Institute of Animal logy, Cambridge (Paterson, 1944) :

Jan.–April	Thousand-head kale and mangolds.
May–June	Fresh grass, spring cabbage or chicory.
July–Oct.	Chicory, savoy cabbage and marrow-stem kale.
Nov.–Dec.	Marrow-stem kale and mangolds.

as possible the same green food is not fed on two successive days.
een food must be fed fresh ; dead foliage should be removed and soil and ashed off. If it is frosted it should be soaked in warm water before it is to the guinea-pigs.

In the U.S.A. it is becoming an increasingly common practi laboratory animals on pellet diets specially prepared for each speci mercial firms. These pellets contain the nutriment requirements in proportion. The pellets are placed in wire-mesh racks, and the ani the pellets at will through the mesh. The method has a number of ad it allows a constant and standard diet to be fed, there is no excretory c tion of the food, little is wasted and labour is saved in that with t racks distribution of food need only take place twice a week. Furt of the method may be obtained in pamphlets by Herrlein (1940) and t Purina Co. (1942). Some of the American diets have been an Slanetz (1943) in respect of protein, fat, certain minerals, vitamin tocopherol, and a number of vitamin-B fractions. Some of the requi guinea-pigs as regards vitamin-B fractions were determined. One or in this country have made up similar diets, although they are not yet e used. So far as guinea-pigs are concerned it seems that in view of t stration that certain grass-juice factors are necessary for adequate n pellet diet should be supplemented with green food.

[*Editorial Note.*—The following references on the nutritional requireme guinea-pig may be quoted also : MITCHELL, W. A., & PLEASANCE, E. (1927), *The Journal*, **6**, 165–169, " On feeding laboratory animals "—gives an account of the method of feeding rabbits and guinea-pigs. PETRIE, G. F. (1930), *J. Hyg.*, 154–164, " An analysis of the influence of irradiation by means of a mercury-va upon the health and fertility of a breeding stock of guinea-pigs and upon the hea offspring during the first six weeks of life." HOGAN, A. G., & RITCHIE, W. *Res. Bull. agric. exp. Sta., Univ. Columbia*, No. 219, " Nutritional requirements and guinea-pigs." The papers on " Grass juice factor," by G. O. KOHLER a leagues at Wisconsin, *J. Nutrit.* (1938), **15**, 445–449, and *Proc. Soc. exp. Biol., N* **40**, 154–157 ; and by CANNON, C. D., & EMERSON, G. A. (1939), " Dietary requi the guinea-pig with reference to the need for a special factor," *J. Nutrit.*, **18** The papers by VAN WAGTENDONK, W. J., WULZEN, Rosalind, and their col Corvallis, on " Effects of milk diets on guinea-pigs," *Amer. J. Physiol.* (1941), and on " A dietary factor essential for guinea-pigs, 1, 2 and 3," *Arch. Bioch* **1**, 373–377 ; *ibid.* (1944), **3**, 305–310, and *J. biol. Chem.* (1944), **155**, 337–343. D. W., & SPRINCE, H. (1944), *J. biol. Chem.*, **153**, 687–688, " Identification of f one of the unknown dietary essentials for guinea-pigs." WORDEN, A. N., & S (1944), *J. comp. Path.*, **54**, 77–87, " Nicotinic acid deficiency in the pig and guin KUIKEN, V. A., MCCOY, R. H., SCHULTZE, M. O., & KING, C. G. (1944), *J. N* 385–394, " Simplified diets for the guinea-pig." A private communication, date 1944, from H. G. HERRLEIN, encloses a brochure containing revised in superseding that quoted in his 1940 publication, on Rockland pellet diet for gu

5. Handling and Marking

Handling. The guinea-pig can be easily controlled by placing the ri over the animal's shoulders with the fingers and thumb around the nec extending the hind limbs with the left hand.

Marking for identification. The usual method of marking guine to stain patches of their hair with various dyes such as gentian viole fuchsin and picric acid. However, the method is suitable only on ligh animals, and in prolonged experiments the staining must be renewed at as the dyes tend to fade.

With parti-coloured guinea-pigs, no two animals of which are mark sketch cards illustrating the colouring of each individual guinea-pig ar some laboratories.

ittner (1941) has described a method of ear-punching in mice which might lapted for guinea-pigs. The mice are marked by a series of holes and ies in the ears. The units are recorded in the right ear, and the tens in eft ear. The position of the holes or notches varies, each position repre- ng a number. By this system it is possible to number mice from 1 to 99. ever, the system may have its limitations in guinea-pigs, who often exhibit ations of the edges of the ears.

6. Anæsthesia and Euthanasia

næsthesia. Anæsthesia is usually accomplished by the administration ier. For anæsthesia of short duration the guinea-pig is placed in a glass ner with a wad of cotton-wool soaked with ether. When prolonged hesia is required the ether must be vaporized by pumping air through it. of the barbiturates given intra-peritoneally might also be used for pro- el anæsthesia. The type of drug and the dosage should be ascertained imentally.

hackle (1944), whose views on anæsthesia in the rabbit are given in Chap- pp. 74–80, states that ether alone is effective for small animals such as a-pigs, which do not require much anæsthetic, and are not unduly dis- d by the induction. Guinea-pigs used for the provision of complement næsthetized frequently by this means without any apparent ill-effect. (1944) advises the use of chloroform 1 part to ether 25 parts. May (1944) mends ethyl chloride as giving a quicker and smoother induction—*Ed.*]

uthanasia. In the destruction of laboratory animals two considerations be borne in mind. First, the method must be humane, and secondly uld not cause changes which are likely to obscure any pathological lesions may need study at a subsequent post-mortem examination. The al principles of euthanasia in small animals have been discussed in a t on the subject published by the National Veterinary Medical Association 7). The administration of chloroform by inhalation is one of the commonest ods of euthanasia used for guinea-pigs. The guinea-pig may be placed r a bell jar which contains cotton-wool soaked in chloroform.

Booth (1944) has noticed that if the lethal chamber is entirely dark the ea-pig remains quiet, whereas if a glass panel allows light into the chamber nimal tends to become frantic and scramble about.—*Ed.*]

Poisoning with coal-gas is a satisfactory method if the gas is given slowly carefully. The animal is placed under an inverted glass funnel which is nected to the gas point by a rubber tubing. If the gas is released slowly the ea-pig gradually becomes drowsy and dies without struggling. Rapid inistration produces struggling and asphyxiation.

The intra-venous inoculation of various toxic agents is not practicable in guinea-pig, but in skilled hands the intra-cardiac inoculation of chloroform, rocyanic acid or saturated solutions of magnesium sulphate causes imme- e death. However, in inexperienced hands the heart may not be punctured death is delayed.

A common method of destruction is by dislocation of the neck. The ea-pig is held head down across the knee and the neck is given a sharp cing downward blow. This method may cause extensive hæmorrhage in subcutaneous tissues of the neck.

7. Disease and its Control

General considerations. One of the greatest problems in the husb laboratory animals is the control of disease. In stocks which are f recruited by outside purchases outbreaks of disease must const reckoned with. In self-contained units disease does not present difficulty, but nevertheless it is always a possibility. The idea that disease-free stocks can be built up is a misconception. Every comm guinea-pigs has its bacterial flora, its masked virus infections, and and endo-parasites. It is not possible, at least so far as bacteria an are concerned, to rear guinea-pigs free from potential pathogen organisms may be brought to light by a variety of exciting circun including sudden changes in diet, violent fluctuations of temperatur animal house, damp bedding, in fact any abnormal condition in the ment of the stock. In many outbreaks of disease it is sufficient to det a " trigger " mechanism and deal with it, and the disease will prove tc limiting. In other infections more drastic measures may be necessary. may range from slaughter of all the affected animals and their in-con vaccination or chemotherapeutic treatment of all the stock. In caused by the ingestion of the causal organism may be controlled by a tion in the feeding methods, so as to avoid contamination of the food.

All food should be kept in vermin-proof metal bins. The anima should be free from wild rats and mice and all species of animal other t laboratory animals normally kept there. It is not practicable to insist purchase of sterilized food, except in the case of pellet diets, so that th bility of contamination of the food and contamination before it reac animal house must be borne in mind.

In the successful control of disease it is essential that there shall be a investigation into the causes of the death-rate. This is best accomplis having one member of the scientific staff responsible for this investigati should be his duty to perform post-mortem examinations on all de culled animals and, when possible, to ascertain the cause of death. If post-mortem service is coupled with routine bacteriological investigati unlikely that an outbreak of disease will have much chance of getting way before control measures can be attempted. Frequent consultation b the scientific worker responsible for this supervision and the head attendant will materially tighten up disease-control. Other members staff who come across intercurrent infections in the course of their exper should inform the worker responsible, so that steps can be taken to tr source of infection.

If an adequate record of deaths and their causes is kept, it will be fou over a number of years certain diseases tend to recur at certain seasons o certain conditions, and it may be possible to forestall them by appr action. In this laboratory a death-rate which can be kept under 2 pe per week of the total stock is regarded as satisfactory.

A brief description follows of some of the commoner diseases of the g pig. No attempt is made to describe each condition clinically since, ex a few conditions, symptoms are not pathognomonic. Experience will detecting guinea-pigs which are not healthy. No details are given of m of demonstrating and isolating the causal organisms of diseases as the

i text-books fulfil this function in a more adequate manner than is possible
hrticle of the present scope.

ratyphoid. This infection is probably one of the most frequent diseases
nea-pigs, particularly in dealers' stocks. The causal agents are members
Salmonella group of bacteria. The most common species obtained is
imurium, although outbreaks have been described in which *S. enteriditis*
ie Reading type were isolated. Infection is by ingestion. The disease
ake one of two forms, the acute form with death in 2 or 3 days, and a
c form which may continue for weeks. Recovery in the latter condition
ccur, the survivors becoming carrier animals. These carrier guinea-pigs
e initiators of fresh outbreaks of the disease when environmental condi-
re suitable and there is a fresh population at risk.

the acute form there are few macroscopic post-mortem lesions. The
may be slightly enlarged. The bacteria can be recovered from the heart
liver and spleen. In the chronic disease, lesions are usually evident.
is considerable enlargement of the spleen, which presents small foci of
s which do not project above the surface of the organ. Greyish-white
necrosis are common in the liver. These foci are often distributed along
border of the liver, they are pyramidal in shape with their bases on the
ry of the organ. When the liver is more extensively involved irregular
f necrosis are distributed throughout the parenchyma, and these lesions
h with the surface. Their appearance is characteristic. The mesenteric
glands are always enlarged and oedematous. In the chronic form
ella cannot always be recovered from the heart blood and spleen.

disease should not be diagnosed without bacteriological evidence, and
sence of lesions does not always imply freedom from infection. All
animals and their in-contacts should be destroyed. In most cases the
actical solution is to kill off the whole stock and start afresh. Attempts
een made to produce immunity by means of vaccines, but the degree of
ity attained has not been sufficient to stop outbreaks. Meticulous
on to hygiene is necessary.

umococcal infection is a common disease. The disease process is
ily an inflammation of serous surfaces. Peritonitis, pleurisy and peri-
s may all be present. The serous surfaces are covered by a thin fibrinous
ith an abundant yellowish-brown exudate. A gangrenous or caseous
onia may also occur. In our experience this disease has quite a marked
cidence, infection being most frequent in females after parturition.
s of the genital tract are common. Abscesses of the uterine wall and the
ligament often appear to be the primary foci, and may be found when
er lesions are present. Pneumococci can be isolated from all these lesions
ay be seen in large numbers in films of exudates.

ntrol measures are difficult. Few premonitory symptoms are present,
eath occurs frequently without any warning. Some laboratories have
pted prophylactic treatment with sulphonamide drugs, but the results
been difficult to evaluate.

eudo-tuberculosis in the guinea-pig is caused by *Pasteurella pseudo-*
ulosis, although it must be realized that a pseudo-tuberculous pathological
s can be caused by certain other organisms. Infection probably occurs
. The *post-mortem* findings may vary considerably, but the mesenteric
glands are nearly always enlarged and caseous. The enlargement of

the mesenteric lymph glands may be very considerable and they ca
confluent. In some cases the liver and spleen may have numerous
scattered throughout their substance. These foci project above the
the organs. Similar nodules may be scattered throughout the
peritoneum. Abscesses associated with Peyer's patches in the smal
are also seen. The lesions contain a smooth thick creamy pus.

Three types of the disease have been described, an acute septica
with rapid death and few macroscopic lesions on post-mortem exa
the well-recognized form with diarrhoea, emaciation and death in 3 o
and a glandular form involving the cervical region.

A considerable amount of work has been done on the antigenic prc
Pasteurella pseudotuberculosis, mainly in connection with cross imn
Pasteurella pestis. Although results were conflicting, there is a possib
a useful vaccine might be produced (Schutze, 1929).

Not uncommonly an infected sow may pass on infection of either
or chronic type to her offspring shortly after birth. If lesions of pseu
culosis are discovered in any unweaned young stock which die, ther
and the litter-mates should be destroyed. This procedure is only pr
when the sows are allowed to litter down in individual hutches.

These three diseases are well-defined ætiological entities. There a
number of conditions having a similar pathology but caused by a v
agents.

Cervical adenitis. The guinea-pig is liable to infection of the glan
cervical lymphatic chain by a number of organisms. Vinzent (1928)
to have been the first worker to draw attention to a cervical adenitis c
a *Streptobacillus*, and Saenz and Refik (1928) reproduced the disease b
food contaminated with pus from the natural lesions. Infection by an
of the *Streptobacillus moniliformis* type has been described by Klie
(1939, 1940) and by Wilson Smith (1941). The disease has occurred
the guinea-pigs at this laboratory (Weitz, 1942). The condition
itself as large swellings in the neck, usually unilateral. These swel
chronic abscesses containing thick creamy pus enclosed in thick fibrous
The disease appears to have little systemic effect, the abscesses fr
undergoing spontaneous retrogression. In other cases the lesions rup
the pus is discharged through an external sinus. Wilson Smith (1941)
that infection may arise through abrasions of the buccal mucosa follo
ingestion of rat-contaminated grain.

Seastone (1939) has drawn attention to a cervical adenitis associa
Group-C streptococci. This condition presents a similar clinical and pat
picture to that caused by the *Streptobacillus*. Infection appears t
ingestion. Seastone observed that an acutely fatal variant of the
strain of Group-C streptococcus occurred.

Cervical adenitis may be present in pseudo-tuberculosis and in so
tions of the respiratory tract.

Infections of the respiratory tract. In some guinea-pigs there ar
signs such as a watery nasal discharge, coughing and sneezing. The
bacteria may be associated with respiratory tract lesions: *Bacteri
lander, Br. bronchiseptica, Pasteurella, Pneumococcus* and *Streptococc*
pathology of these infections has been described by Keegan (1920).
outbreaks there is an association with conditions of overcrowding,

dity or damp bedding. The correction of these defects may overcome the d of infection.

rus infections. It is well recognized by virologists that experimental can be vitiated by failure to realize that the animals used may already be ly infected with a virus different from the one inoculated. Although a few virus diseases of guinea-pigs have been recorded, undoubtedly s will be brought to light as work on viruses continues.

omer (1911) described an **infectious paralysis** which occurred sporadically. was a gradually increasing muscular weakness, particularly of the hind ers and limbs. In some cases there was paralysis of the bladder. Patholy the process was a disseminated meningo-myeloencephalitis.

e **Salivary gland virus** of Cole & Kuttner (1926) is widely spread in adult a-pig stocks. It appears to cause no clinical symptoms in naturally-ed guinea-pigs, although it causes death when inoculated intra-cerebrally oung guinea-pigs. [Pappenheimer, A. M., & Slanetz, C. A. (1942), *p. Med.*, **76**, 299–306, have described "A **generalized visceral disease** of ea-pigs, associated with intranuclear inclusions" which they could not smit satisfactorily from spontaneously-infected to other animals and which believe to be distinct from salivary gland virus infection.—*Ed.*]

An **epizootic hepato-enteritis** caused by a virus has been described by os (1933).

Coccidiosis. Lapage (1940) has made a study of the disease in guinea-pigs compiled a summary of the literature. The only species of coccidium cting the guinea-pig is *Eimeria caviæ*, and infection is widespread. Visible s of illness are diarrhœa, contraction of the buttocks under the body, ked loss of appetite and polyuria. Mucus may be passed in the fæces. ever, there may be a very heavy infection of the bowel without clinical ptoms, and apparently little tissue damage.

Although coccidiosis may take on epizootic proportions, especially in young ea-pigs, it is not usually a cause of death. Death, when it occurs, is usually other complicating factors. Lapage was unable to produce coccidiosis by ing inadequate diets. Control of the disease must be on hygienic lines.

Editorial Note.—Other references which may be quoted include those on **Spontaneous rculosis,** by Cummings, D. E. (1932), *Amer. Rev. Tuberc.*, **26**, 388–407; by Saenz, 932), *ibid.*, 437–439; by Calmette, A. (1933), *Ann. Inst. Pasteur*, **50**, 148–160; and otchin, E. (1944), *Vet. Rec.*, **56**, 437. On **pneumococcal disease** by Neufeld, F., inger-Talczyiska, R. (1932), *Z. Bakt.*, I. (Rep.), **109**, 52–53; and by Petrie, G. F.), *Vet. J.*, **89**, 25–30. On **streptococcal infection** by Smith, T. (1931), abstracted *t. Bull., Weybridge*, **2**, 425, from *Internat. Clin.*, **3**. 276–297; and by Rae, M. V. (1936), *fect. Dis.*, **59**, 236–243. On ***Salmonella enteritidis* infection,** by Duthie, R. C., & hell, C. A. (1931), *J. Amer. vet. med. Ass.*, **78**, 27–41; and by Jungherr, E., & tridge, W. N. (1933), *J. infect. Dis.*, **52**. 413–419. On "An **epizootic septicæmia ung guinea-pigs caused by *Pseudomonas caviæ* n. sp.**"; by Scherago, M. (1937), *fect. Dis.*, **60**, 245–250. On "**spontaneous toxoplasma encephalitis** in the guinea- by Markham, F. S. (1937), *Amer. J. Hyg.*, **26**, 193–196; and on "A case of aneous toxoplasmosis in a guinea-pig," by Boiseau, R., & Nodenot, L. (1936), *Soc. Path. exot.*, **29**, 135–141, cited in *Vet. Bull., Weybridge*, **8**, 571. On "A **derma-cosis** of guinea-pigs," by Shaman, M. S. (1937), *Arch. Derm., Chicago*, **36**, 335–341. On **"A highly fatal disease of guinea-pigs,"** by Ten Broeck, C., & Nelson, J. B.), *Proc. Soc. exp. Biol., N.Y.*, **39**, 572–573. An English translation of Prof. H. iger's (1933) textbook on *The Guinea-Pig: Breeding, Keeping and Diseases*, prepared r. A. L. Wilson, M.R.C.V.S., is in UFAW's possession and will be loaned on application.

Some incidental references to cases of disease in guinea-pigs, e.g. to listeri given in Chapter 4, on the rabbit.]

The writer would like to express his thanks to Mr. W. Moss, animal attendant at this laboratory, for so readily placing his great ex and knowledge of guinea-pigs at the writer's disposal.

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er references are cited editorially at the end of the sections on nutrition and on
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CHAPTER 6

THE NORWAY RAT

(*Rattus norvegicus*)

Compiled from various sources,
with a section on diseases

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and with an Appendix on Wild Brown Rats

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1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breed
5. Handling and marking. 6. Anæsthesia and euthana
7. Diseases in the Laboratory Rat. Appendix: Wild brow

1. Introduction

The albino and hooded (black-and-white or chocolate-and-white) c
cated varieties of *Rattus norvegicus* (the brown rat) have been more ext
used than any other species of laboratory animal. The albino vari
recently formed the subject of an extensive monograph (Griffith &
1942), in which thirty contributors have provided valuable chapte
breeding, management, gross anatomy, embryology, dietary requireme
teeth, the digestive system, metabolism, central nervous system, inves
of psychological phenomena, circulatory system, use in hormone assay
of drugs, hæmatology, radiology, surgery, histological methods, the
system, the eye, protozoan parasites, metazoan parasites and spon
diseases. This publication supersedes that of Donaldson (1924) and
be available in all laboratories in which the rat is employed for exper
work. This monograph also contains notes on the management of a s
wild grey Norwegian rats which has been maintained at the Wistar I
for many years (King, 1939).

The information in the present chapter is essentially of a practical
and is to a considerable degree based upon methods in use at the Div
Nutrition, Lister Institute of Preventive Medicine, London, S.W.1, w
colony of piebald rats was obtained in 1923 from Dr. Gladys Hartwell
Department of Physiology, King's College for Women, London, and exc
some early additions from the parent stock has since been maintai
inbreeding. An account of some of the features of general managemc
feeding-methods developed during the early years of the colony was gi
Henderson-Smith & Chick (1926). This colony has been utilized
successfully for nutritional work and for certain types of pathological
Genetical records have not, however, been kept.

The main features of this and other successful rat colonies are scru

ess, strict attention to environmental temperature and related factors, te nutrition, and painstaking care in general management. Great laid upon personnel. Hume (1945) regards choice of the right technical it (preferably female), " and the training of that right person by another rson " as by far the most important feature in the management of any ny.

2. Accommodation

e rat rooms. The details of room-construction, ventilation, heating-, lighting, hot-water supply, cage-racks, washing-up accommodation tensils, sterilizing plant, drinking-water supply, balance, and food-ration room or annexe, given in chapter 2, are applicable to the rat. The g and ventilating systems should be adjusted to maintain a temperature- not exceeding 65° to 70° F. (18·3° to 21·1° C.), and there should be a um of draughts but good ventilation. Wood (1944) advocates a tem-re of 70° C. in the experimental rooms and one of 65° C. in the breeding- A rat room becomes oppressive if the ventilating system is inadequate, here in an unsuitable type of room the choice is between two evils, siveness must be endured in order to secure a constant temperature absence of draughts. Relative humidity should fall within the range of 0 per cent. throughout the year.

separate room for breeding is desirable, and whenever possible different of experimental work should be carried out in different rooms. Where imal unit is not self-contained, great care should be taken to avoid g in transit from breeding to experimental room, or between inoculation maintenance rooms. When necessary the caged rats should be trans- by means of a trolley, fitted with draught screens, along corridors which aintained so far as possible at the same temperature as the animal rooms . Weighing, handling, marking and infecting can all be carried out in a te portion of, or in an annexe to, the breeding or experimental room, but vironmental conditions must approximate as closely as possible to those ch the rats are bred and maintained.

ke other murine rodents, rats like facilities for shelter. Where shelters the cages cannot be provided (see p. 117 below), it is therefore desirable mmer months to ensure reasonable hours of darkness in the rat rooms.

hen he has found it necessary to keep rats (or guinea-pigs) in the [bio-cal] laboratory rather than in the animal house, Greville (1944) adopts llowing procedure. The animal is kept on the usual wire grid of the cage he cage-bottom is covered with a sheet of newspaper. On the newspaper tered a thin layer of powder of the following composition :

Talc	..	..	1 oz.
Kaolin	..	..	20 oz.
Thymol	..	..	46 grains.
Vanillin	..	..	30 grains

owder absorbs most of the urine and the latter is prevented by the anti- from decomposing with release of ammonia into the atmosphere. The n provides what is usually regarded as a pleasant smell. Every morning, re often if preferred, the paper is pulled out of the cage bottom : it peels cleanly with all the excreta.

Cages. Apart from special types, e.g. those employed in metabolis exercising, rat-cages are usually of three types, viz. (1) large cages empl mating, for housing resting does, or as nurseries for weaned animals selected for breeding or experiment; (2) smaller breeding-cages i individual does may give birth to and suckle their young; and (3) c maintenance during experiment. The rat is usually employed for r long-term studies. Cages of type (3), therefore, should be as large as patible with available room and labour, and should be comfortable. W they will not interfere with the experimental design or with the system of therefore, both shelters and exercising devices should be fitted. The mental cages at present in use in most laboratories are not to be rega

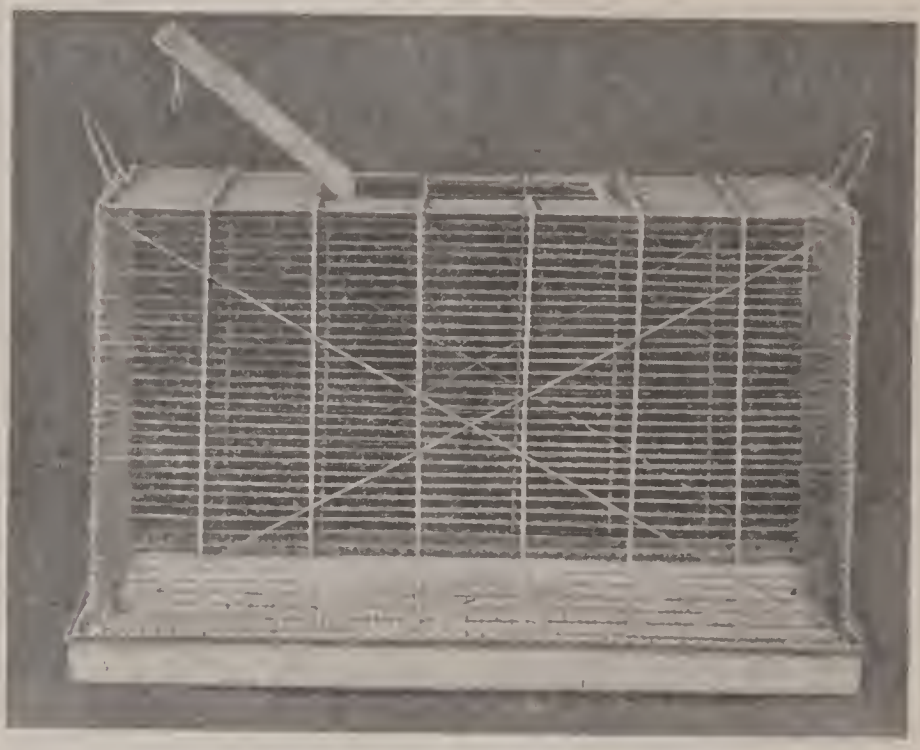


FIG. 25.—LISTER DESIGN OF LARGE CAGE FOR GROUPS OF RATS.

ideal (see Chapter 1, p. 19), but those illustrated here have each advantages incorporated as the result of experience.

All-metal cages only are described in this chapter. At the Wistar I wooden cages have been selected in preference to metal ones, the reason being simplicity of construction and absence of rust (Farris, 1942, p. 7). are claimed to be odourless, and they may be painted with lice-repellen (Farris, 1942). The need for metal cages is, however, suitably stre Chapters 2 and 3 of the present handbook and pending the introducti suitable plastic cage we feel bound to recommend all-metal cages for the housing of all laboratory murine rodents. Such cages should be galva otherwise treated so as to reduce rusting. In certain experimental stances, e.g. in mineral work, cages of glass or other non-metallic const may have to be employed.

The cages illustrated here have separate trays or pans. Where batt cages are employed, however, the method advocated by Farris (194 might well be adopted. In this method pans are omitted and a 40-lb.

Kraft paper or of a similar paper is mounted at the end of each row of The paper is passed under the wire floor of the entire row of cage-, and " cleaning is accomplished by withdrawing the soiled paper from h the cages at the opposite end of the roll stand. This automatically clean paper." The method is illustrated in figure 7 of Farris's article.

ges to house groups of rats. The Lister design of large cage for mating or rats or for groups of growing rats consists of an inverted box-shaped of wire mesh, resting on a perforated zinc tray containing peat (Fig. 25). ame measures 30 in. *l.* × 18 in. *b.* × 18 in. *h.*, and its wire mesh is between uprights and $\frac{1}{2}$ in. between cross-wires. The top is flat and ns a wire trap-door measuring 10 in. × 10 in. Wood-wool, changed at

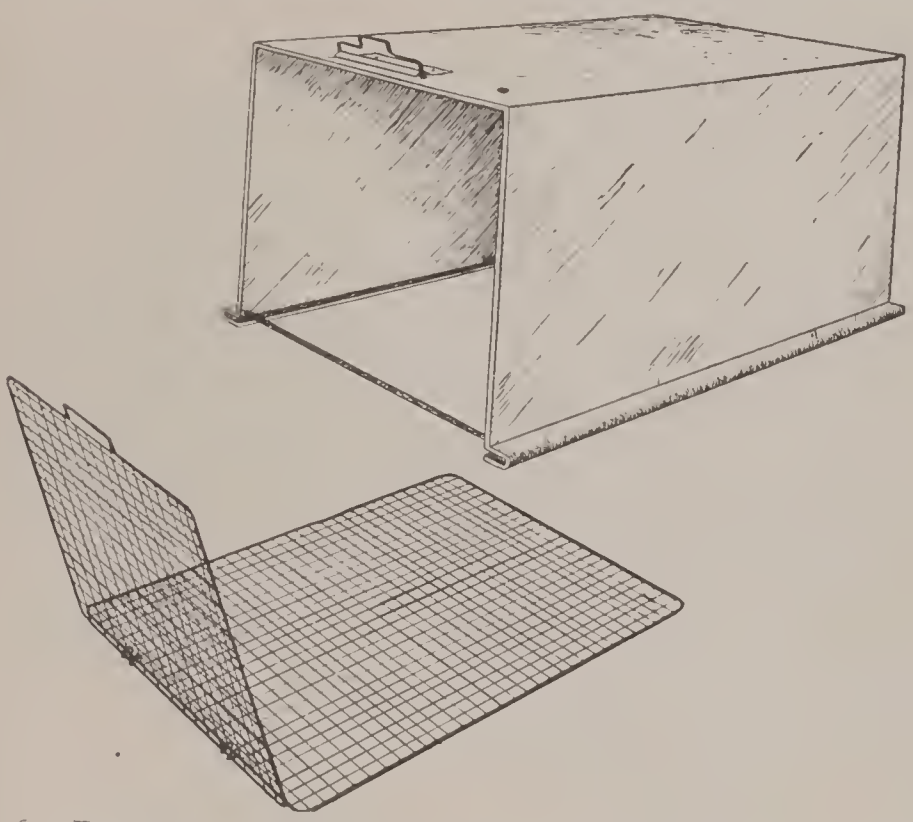


Fig. 26.—Breeding-Cage, with detachable Wire Floor and Door.
Animal Research Laboratory, Lever Bros. & Unilever, Ltd.

vice weekly, is a suitable bedding material for cages of this type and if possible, be sterilized before use. Simpson (1944) has encountered from wood-wool infested with fly-eggs and maggots. The flies hatch a week or ten days and may form a veritable plague in the summer unless the bedding is changed frequently. Wood (1945) attributes outbreak of lice in his colony to infested wood-wool with which wild d been in contact.

eful type of cage for mating and similar purposes is that in use in the Research Laboratories of Lever Brothers & Unilever, Ltd. (Tainsh, nd illustrated in Fig. 26. The top and three sides are made of sheet The floor and the full-opening door, both of wire-mesh, are hinged to form a unit that can easily be detached for cleaning. The floor rted in two slots in the side walls. A simple clip allows the door to ed or closed with one hand. Each cage measuring 24 in. *l.* × 12 in. *b.*

× 12 in. *h.*, is housed in the angle-iron castered stands illustrated in twelve cages to a battery.

Breeding-cages. Small cages in which individual does are place pregnant and left until their young are weaned, are at the Lister Institu on the box-and-lid principle. They are of perforated zinc throughout, ensuring privacy for mother and young, and facilitating the conserv heat. The measurements are 20 in. *l.* × 11 in. *b.* × 7 in. *h.* The cage

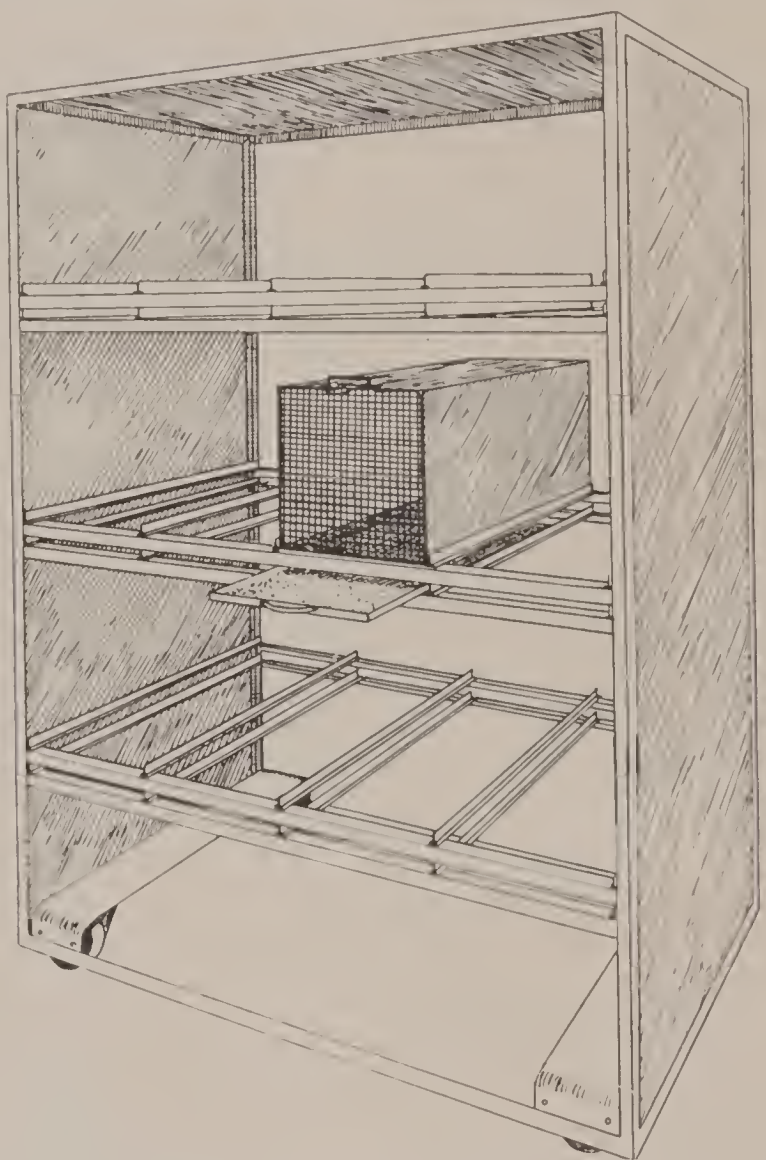


Fig. 27.—Battery to House Twelve large Cages.
Animal Research Laboratory, Lever Bros. & Unilever, Ltd.

individual trays containing peat, and are supplied with abundant wo for bedding and nest material.

In the Lever Brothers & Unilever laboratory (Tainsh, 1944), a version of the cage illustrated in Fig. 26 is employed for does from the pregnancy diagnosis to weaning. This version is employed also for litters and measures 18 in. *l.* × 9 in. *b.* × 8 in. *h.* Twenty-four su are housed in the castered stand illustrated in Fig. 27.

war (1945) employs a small version of the semi-closed cage illustrated in
o for pregnant animals, in preference to the box type. The wire grid is
ed but a large handful of wood-wool is added. Results have been good.

ges for maintenance during experiment. Where groups of rats are main-
together, the type of cage depicted in Fig. 25 is suitable for experimental
In most experimental work with rats, however, animals are maintained
lually, often for relatively long periods. Every effort should be made to
that the animals are comfortable, have room for exercise and are not
Fraser (1944) and others stress the appreciation by rats and other
rodents of something to gnaw. C. W. Hume, on the basis of experience
et rats, suggests the provision of a hard piece of wood where this would
terfere with the experimental conditions.

r nutritional work with the B vitamins a grid floor is essential to reduce
hagy. Provided the room is adequately warmed and free from draughts,
bottom of wire mesh to each cage is of great advantage for almost all
mental work with rats. It makes it much easier to keep the cage clean
educes helminth and other
tary parasitism to a mini-
The objection that rats may
comfortable on such a floor
evelop foot-sores can be met
t in part by the provision of
g, when the experimental
ons permit, or of a small
m of metal sheet (preferably
a simple shelter). A Petri
also suitable for resting
es (Simpson, 1944). Rats
t readily foul such separate
g-quarters.

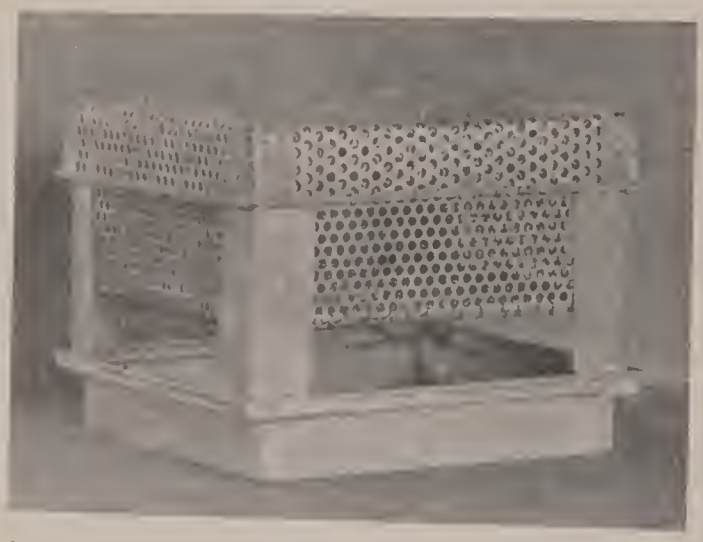


FIG. 28.—LISTER CAGE USED FOR VITAMIN B EXPERIMENTS.

type of cage employed for
-B experiments at the Lister
te is shown in Fig. 28. It
es 11 in. *l.* × 11 in. *b.* ×
overall, and rests, supported on angle-shaped legs, in a zinc tray. The
is not false but made of the same wire mesh as the sides. The lid
chable. The whole cage is changed frequently during the course of an
ment.

the Lever Brothers & Unilever, Ltd., laboratory (Tainsh, 1944) a
× 9-in. *b.* × 8-in. *h.* version of the cage illustrated in Fig. 26 is
ed for individual experimental rats. Forty-eight such cages may be
in the castered stand illustrated in Fig. 27.

another type of experimental cage used for individual rats is that
d for the Orkney vole by Dr. J. A. Young (see Fig. 44, p. 193). With
ent dimensions this cage is used without its nesting-box for a rat. A
ersion might well include the nesting-box and be suitable for breeding
many types of experimental work.

Lings.

er-bulbs, as described in Chapter 2 (see Figs. 14 and 15), are suitable for

routine use with rats and are strongly recommended in preference to bowls. Clean water should be provided daily. The automatic watering employed in the Wistar Institute rat colony is referred to on p. 36.

Food-receptacles may be of various types. In the type of cage emplo the Dunn Nutritional Laboratory, Cambridge, the food-pot is clipped int Herrlein (1940) strongly recommends the use of wire-mesh food rece

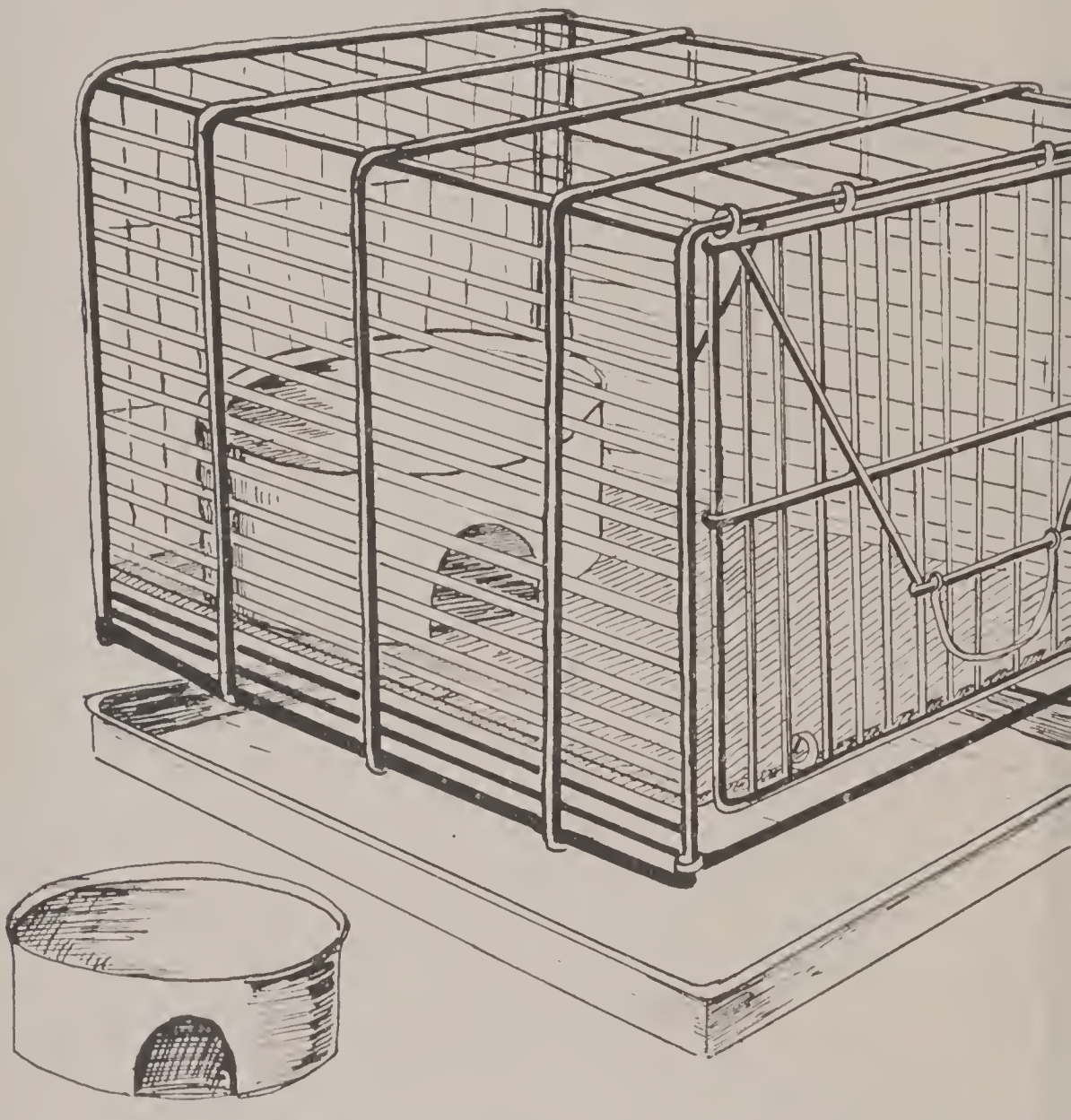


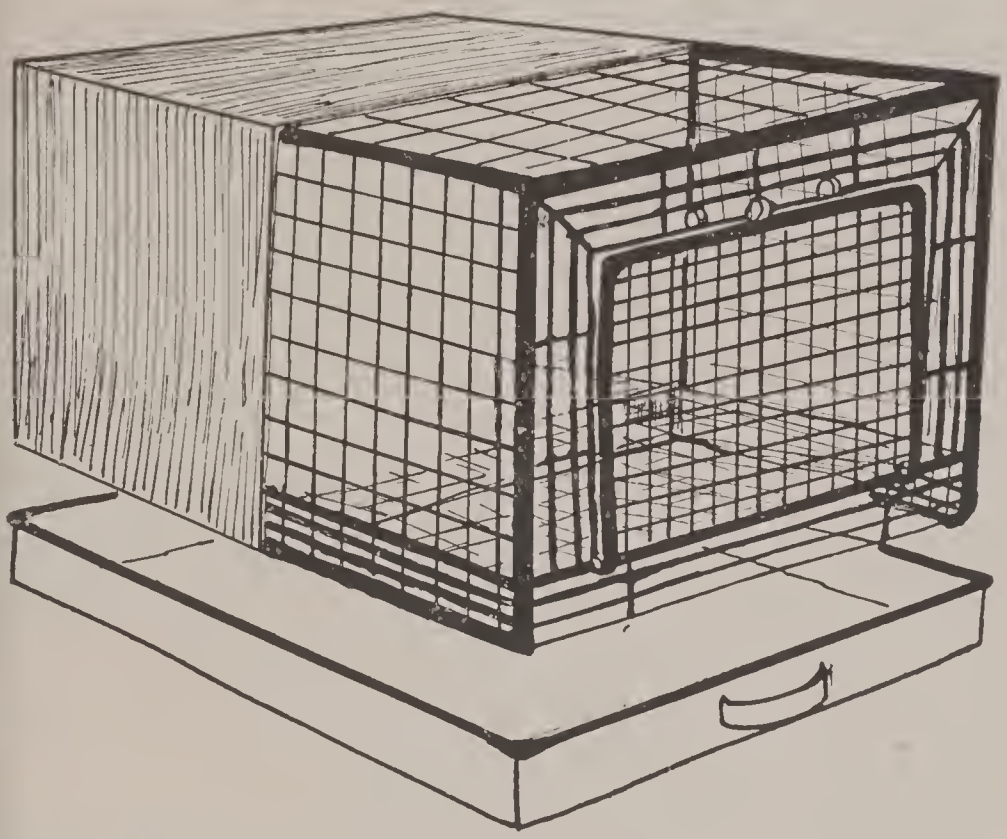
FIG. 29.—HUT USED BY DEWAR TO PROVIDE SHELTER FOR RATS.

The illustration shows the hut in the open type of cage used in the B.D.H. Lab London, N.W.1. The dimensions of this cage are as given in Fig. 30.

suspended at least 3 to 4 in. from the floor of the cage, and used in con with solidified food. By such methods he aims to reduce materially the of contaminating the food, and so assist the control of bacterial infectio rats a $\frac{1}{2}$-in. mesh is recommended. As Herrlein stresses, it is import food should never be exposed to contamination with fæces or urin another type of food-receptacle is that used with most metabolism cage

fixed outside the cage proper and the (individual) rat can gain access to d but has difficulty in scattering it. Even if it does succeed in scattering, od usually falls outside the cage proper and can be collected on a sheet of or paper placed for the purpose. The feeding-device in use at the Institute for any diets is illustrated by Farris (1942, p. 10).

speed in routine management of the colony, free food receptacles have advantages. Such receptacles should, however, not be easy for the overturn, should be so constructed as to prevent excessive scattering of great nuisance in nutritional experiments, particularly when attempts ng made to estimate food-intake), and should be easy to clean. A food- the non-scattering type is illustrated in figure 15 of the article by Farris



IG. 30.—SEMI-CLOSED TYPE OF CAGE USED AT THE B.D.H. LABORATORIES.
made in two sizes, viz., 17 in. × 12 in. × 10 in., and 17 in. × 8½ in. × 6 in.

Wood (1944) formerly employed suspended round-bottomed spun- um feeding-bowls, but found that these wore out mainly as a result of lowly chewed away by the rats in moments of boredom. He now uses mber glass jars with upright sides. These jars are of the type used for intments and shaving creams, and a supply of rejects, having *external* as obtained cheaply and found to be quite satisfactory.

feeding during critical microbiological experiments, some modification eed-tube usually employed for mice may be employed.

e form of *shelter* is advocated in open cages of the usual experimental Dewar (1945) employs "huts" made out of cake tins (Fig. 29). The e of two sizes—diameter 9 in., height 3 in., or diameter 6 in., height 3 in. sirability of providing such shelters has been discussed in Chapter 2.

Where wire-mesh floors are employed, the rest (e.g. Petri dish) mentioned al may be placed beneath the shelter.

Some form of *exercising device* is a great advantage, especially for indivi rats on long-term experiments. The main obstacle to the usage of such dev is the fact that they may clutter up the cage and so hinder routine managen and cleaning. The ideal remedy is the employment of a larger cage. E cising wheels are illustrated in Fig. 13, p. 36. Modifications of exerci wheels suitable for activity records, and exercising cages proper, are descr and illustrated by Farris (1942, pp. 9–10).

Detachable metal *label-holders* and other cage fittings are discusse Chapter 2, pp. 28–38.

It is not the purpose of this book to describe special types of experime cage, except in so far as the management of the animals is concerned. *obstetrical cage*, designed to record automatically the time of birth of the yo is described by Long & Evans (1922).

Cage routine (changing, cleaning and sterilizing). A strict cage rou should be enforced. Outside the Wistar building there is a large st sterilizer, and all bedding, shavings and stored cages are sterilized before b taken into the colony (Farris, 1942, p. 5). Similar practices are adopte many bacteriological laboratories, but they should, wherever practicabl extended to all small-animal colonies. Sterilizing is not a substitute routine washing, and care must be taken to ensure that dirty cages are not pa back into circulation merely because they have been sterilized. When possible, all experimental cages should be changed, cleaned and sterilized weekly. Even when labour is scarce the interval should not be very m greater. For short-term microbiological and similar experiments it is, of co usually desirable to leave the animal in the cage throughout the experim In nutritional experiments involving diets rich in sugar, cleaning and steril more frequently than once weekly may be necessary if the rats are to rema a clean condition. Cages which become soiled for any reason should, if poss be changed immediately. Larger breeding or nursery cages can be cleaned sterilized after, say, the last of the batch of does has been diagnosed as preg and removed to her individual cage. The individual small breeding-cages of course, left undisturbed so far as possible between introduction of the nant doe and removal of her litter three weeks after parturition. The prov of ample bedding and the automatic or manual removal of fæces will assi keeping such cages clean.

3. Nutrition

Dietary requirements. The specific dietary requirements of the rat been more intensively studied than those of any other animal. R summaries include that of McCoy (1942). All important advances are not *Nutrition Abstracts and Reviews*, published quarterly by the Imperial Bu of Animal Nutrition, Rowett Institute, Bucksburn, Aberdeenshire. Fo sake of completeness it may be mentioned here that the Norwegian rat rec ten amino-acids, viz. phenylalanine, histidine, *iso*-leucine, leucine, tryptop methionine, lysine, threonine, valine and arginine (see the series of pape W. C. Rose and collaborators in the *J. biol. Chem.*, 1938, **123**, 39–43, and e references) ; the essential fatty acids, which appear to be best supplie linoleic acid and next by linolenic (see Smedley-Maclean, 1943, Chapte

ium, phosphorus, magnesium, sodium, potassium, chlorine, manganese, , copper, iodine, zinc and probably cobalt (see Shohl, 1939, as well as McCoy, 2) ; and vitamins A, B_1 (thiamin, aneurin), B_6 (pyridoxin), riboflavin G, lactoflavin), pantothenic acid (filtrate factor, probably equivalent to B_3), ine, inositol (Bios I), *p*-aminobenzoic acid, biotin (Bios IIb, vitamin H, nzyme R, required at least under certain conditions), several unidentified nbers of the vitamin B_2 complex, D, E, and, in certain circumstances, K Rosenberg, 1942). There is no conclusive evidence that the rat requires ninium, boron, fluorine, nicotinic acid or amide (pellagra-preventive or anti-ktongue factor, niacin, probably equivalent to vitamin B_5), or vitamin C orbic acid, cevitamic acid, anti-scorbutic vitamin). Vitamin B_4 is no longer rded as an entity. The qualitative requirements of the Norwegian rat for gy-providing foods and for the specific dietary essentials mentioned will be d in the literature quoted.

For refection in the rat see Kon, Kon & Mattick, 1938 ; Kon, 1945.

Practical feeding. Various successful methods of stock-feeding are in use ifferent rat colonies. In general such methods fall into two groups, viz. e in which individual foods or mixtures are given and those in which pound cubes or pellets form the basal portion of the dietary. For nutri-al research it is often necessary to modify the diet with season or with urition, lactation and the post-weaning phase, in order to produce experi-tal rats which do not possess high bodily reserves of some particular dietary or. A regular feeding-time should be observed.

Examples of methods which fall into the first group are those employed at Lister Institute and at the National Institute for Medical Research. At Lister Institute the daily feed for the breeding and nursery stock is given ng the early afternoon, and is normally varied twice weekly by the inclusion dditional animal protein, viz. (1) herring or other fish—sometimes cod in ner—each Wednesday ; (2) raw bullock's heart each Friday. The inder of the ration is composed of bread, soaked either with hot water or the water used for washing the meat or fish, yeast, wheat germ, and fish-on the days on which meat or fish is not fed. A quantity of mixed grains ium bran, rolled oats, wheat, hempseed) sufficient to make a stiff mixture dded. Milk also is fed daily. In order to avoid undue variations (for tional research) between summer and winter, part of the milk is recon-ted from a winter-dried milk (Glaxo). Cod-liver oil supplement is given sionally, except when rats are being produced for work on vitamin A. for vitamin-A work are placed on a diet deficient in this vitamin as soon ney are old enough to feed themselves. Modification of the dietary ction of the yeast fraction) is made when rats are being produced for on the vitamin-B complex.

offey (1944), who employs a bread-grain-greenfood type of diet, with lements of cod-liver oil and Marmite, recommends weekly dosage with nin-E oil. On Saturday mornings each fasting rat is given 2 ml. of nin-E oil well mixed with 10 ml. milk.

ertain foods should be avoided when the above type of feeding is practised. Simpson (1944) has found that too coarsely ground maize, which is mely hard, causes ulceration and bleeding of the forestomach of newly-ed rats. Hartwell (1944) has mentioned the dislike shown by rats for s and potato peelings.

Examples of the cube or pellet method are those employed at the Row Institute (Thomson, 1936), at Lever Brothers & Unilever, Ltd. (Tainsh, 19 and at the Rockland Farms, New City, New York (Herrlein, 1941 ; Arc Farms Milling Co., 1944) ; and at the National Institute for Medical Resea (Parkes, 1946).

At the Rowett Institute (Thomson, 1936), the following mixture adopted after trials :

	per cent.
Wheat offal (fine middlings No. 2)	19·2
Ground wheat	19·2
Sussex ground oats	19·2
Ground barley	9·5
Ground maize	9·5
Meat-and-bone meal (45 per cent. protein) ..	9·5
Dried skim milk	7·0
White fish meal (60 per cent. protein) ..	4·7
Dried yeast (40 per cent. protein)	1·2
Sodium chloride	0·5
Cod-liver oil	0·5

This diet has calculated food values, in percentages, of digestible protein, 14·9 ; digestible protein equivalent, 15·6 ; starch value, 6 calcium, 1·18 ; and phosphorus, 0·99 (Ca/P ratio of 1·19:1). The mixt finely ground, is made up in quantities of about 10 cwt. and compressed ½-in. cubes in a food-cubing machine. The cubes are placed in a corner of cage and are fed on weekdays, together with approximately 5 g. of green f usually kale, and 10 ml. separated milk per growing rat. The number of c required per rat varies with age and sex, but excess is easily detected. G food is regarded as essential for good results. On Sundays food is limited mixture of whole wheat, oats and barley, and dried cooked maize. In albino (Glaxo) and hooded (Lister) rats so fed, the average individual we at 23 days of litters reduced to eight in number at birth is 43 g. for male 41·5 g. for female, figures which, as Thomson points out, compare favour with those published by Donaldson (1924), Simmonds (1924), Slonaker (1 Greenman & Duhring (1931), Mapson (1932), Kellerman (1934), and (1934). The advantages of the cube method of feeding listed by Thomson (1) simplicity ; (2) economy, as the food cannot be scattered as mash so is, and is not readily fouled ; (3) that the cages are more easily cleaned ; (4) that the rats appear to enjoy holding the cubes in their "hands." cubed diet is manufactured for sale by the North-Eastern Agricultural C erative Society, Ltd., Bannermill Place, Aberdeen. During war-time difficulty was encountered in obtaining the same quality of wheat midd as formerly, and their actual percentage formula [quoted by Falconer (1 is : wheat middlings, 38·4 ; Sussex ground oats, 19·2 ; ground barley, ground maize, 9·5 ; meat-and-bone-meal, 9·5 ; skimmed milk powder salt, 0·4 ; dried yeast, 1·3 ; fish meal, 4·8 ; and cod-liver oil, 0·4).

A cube used by Wood is made by Messrs. Thorley, Ltd., King's C London, N.1. In this cube 2 per cent. of molasses is used for binding, and skimmed-milk powder has been increased to 14 per cent. at the expense o middlings ; otherwise the formula is the same as that given by Thomson.

In making up and employing rat-cubes of this type, care must be take

ure that one constituent does not deteriorate in quality. Such an experi-e is recorded by Wood (1944), who detected the change as a result of careful tine monthly examination of weight records.

Other formulæ for cube or pellet rations which may be mentioned include t quoted by Tainsh (1944) and that of the Rockland rat diet prepared in U.S.A. Tainsh's mixture is a war-time substitute and comprises, in per-tages, fine bran, 34 ; broad bran, 8 ; coconut-cake meal, 16 ; ground-nut e meal, 5 ; yellow maize, 11 ; skimmed-milk powder, 6·5 ; dried brewer's st, 5 ; wheat germ, 2 ; meat or fish meal, 2·5 ; dried flaked liver, 2·5 ; e flour, 1 ; limestone, 1 ; common salt, 0·5 ; and molasses, 5. A small ntity of vitaminized oil to provide 8 I.U. of vitamin A and 1 I.U. of min D per g. of diet is incorporated. Slight modifications have to be made n time to time in dependence upon the availability of the ingredients. The ture is ground, steam heated, and extruded through a die which turns out . pellets, of the type used for cattle food, averaging 5 g. in weight. The asses form the binding agent for this process. As with Rowett cubes, en food and milk also are fed, while nursing mothers and young stock are en pieces of ox-heart when this is obtainable. Tainsh records that the nge-over of feeding practice to the pellet system has been accompanied by gnificant lowering of pre-weaning mortality, including infanticide. For the paration of young rats for vitamin A deficiency experiments, the vitamin A plement is excluded from the pellets.

The Rockland complete rat diet comprises ground maize, ground hulled ey, ground hulled oats, ground whole wheat, meat scraps, powdered whole , alfalfa leaf meal, soy bean meal, wheat bran, wheat middlings, pulverized , gluten feed, fish meal, peanut flour, linseed, maize-oil meal, NaCl (not zed), precipitated chalk ($CaCO_3$), cod-liver oil. The average analysis is n in percentages as protein, 25·10 ; fat, 5·46 ; fibre, 5·95 ; carbohydrate, 9 ; moisture, 9·80 ; nitrogen free X, 42·44 ; ash, 11·25. The detailed eral and vitamin contents are given as : Si, 0·59 ; Cl, 0·96 ; Na, 0·88 ; ·70 ; Ca, 2·56 ; P, 1·04 ; S, 0·24 ; and Mg, 0·28 (all per cent.) ; Mn, 5·00 ; 37·00 ; Zn, 2·00 ; and Cu, 0·27 (all mg. per 100 g.) ; vitamins A, 475–I.U. ; carotene, 0·42–04·5 μg. ; B_1, 228–465 μg. ; riboflavin, 538–652 μg. ; 209–275 μg. ; pantothenic acid, 1,989–2,568 μg. ; choline equivalent, ·492 μg. ; nicotinic acid, 14·4 mg. ; C, 5·0 mg. ; E, 7 mg. (all per 100 g.).

Wood (1944) makes up all diets deficient in some specified vitamin or other ient factor either in a form which permits of their being baked into a thin , resembling Yorkshire pudding, which can then be broken up and given in es, or else in powder form, to be given as such in a food pot of the type ribed above.

See the important additional references cited on p. 137.—*Ed.*]

4. Breeding

There is an extensive literature dealing with the breeding and the sex crinology of the rat. Some valuable practical details are summarized by is (1942) and in the notes on " Breeding and parturition in the albino rat " ediately preceding Farris's article in the same monograph.

The œstrus cycle of the rat occupies a little over four days, while both nancy and lactation are of about three weeks' duration. Combined with h rate of fertility, these facts render it possible to plan for the even pro-on over most or all of the year of large numbers of animals. The planning

of such a programme, however, involves considerable forethought and atte tion, and its supervision is usually best left to a thoroughly reliable techni assistant who has made this branch of laboratory work his or her career. substantiation of opinions expressed elsewhere in this volume, the need adequate salaries for persons of this type is stressed. It is unreasonable expect this most important task to be carried out at a rate of pay considerab below that of junior technicians engaged in chemical or pathological work.

The actual breeding systems adopted in different laboratories vary som what in detail, but in all successful cases the general principles are the sam viz. careful selection of breeding-stock, the maintenance of a strict, kindly a hygienic routine, good feeding and accommodation, avoidance of too ea matings, curtailment of litters to a uniform number, prompt culling of unsa factory parents and offspring, and restriction upon overbreeding.

Quite apart from its genetical interest, the careful keeping of records is immense value [See p. 139]. Wood (1944) keeps an individual card for ea buck or doe used for breeding. The animal's own date of birth, age and weig at mating, etc., are noted, and then follows a full mating history—date of pa ing, date of parturition, number in litter, aggregate weight of litter at birth, a so on. Litters are weighed in bulk, not only at birth but also at 7, 14 and days, and again at weaning, if this is later than the 21st day. Individ weights are recorded before using animals for breeding or experimental wo although obviously abnormal individuals are discarded as soon as they noticed. From these records, monthly statements are prepared which are fou to be of practical value in breeding and in other ways, as indicated abo Breeding pairs are carefully selected on their pedigree and previous history. Wood states : " It is obvious that a doe which casts numerically small litters whose mother had this fault, should not be used ; it may not be so obvious t numerically large litters are equally dysgenic, since the individual pups ar low birth-weight and do not in our experience make up this handicap eve the litter is reduced to ten. An ideal breeding-colony would produce as m rats as possible with weights as high as possible ; therefore we select breeding those animals whose own and whose parents' histories show a sa factory weight of pup at 21 days, without the production of litters which too small (under 6) or too large (over 10)." Some laboratories cull at bi or on the second day of life. Wood prefers to cull at seven days as it is t much easier to pick out the runts and it frequently happens that one or tw the poorer specimens die in the first week so that culling becomes unnecess At the Lister Institute it was formerly the practice to reduce litters to 1 summer and to 8 in winter, but better results have followed since the num was reduced to 8 throughout the year. Hartwell (1944) and others rec each litter to 6. Since the sex can be judged at birth, not only records of male : female ratio but also selection of survivors can be made in accord with current experimental and breeding programmes. Other factors w are used by Farris (1942) in the selection of breeding stock include a " stre lined " appearance, not plump or fat, freedom from all external defects, a uniform, smooth and glossy coat.

Potential breeders are usually kept, the sexes being separated at or s after weaning, in nursery cages, e.g. the type illustrated in Fig. 24. Males females used are of approximately the same age and should be between 100 120 days of age at the first mating (Farris, 1942, p. 1). The number of ani

the breeding-cages is varied in different colonies. At the Lister Institute oes and 2 bucks or 5 does and 3 bucks are run together for up to three weeks. the Wistar Institute (Farris, 1942, p. 1) three does and one buck are put in reeding-cage on Monday morning, and the male is removed on the Saturday l replaced by another buck on the following Monday ; this second buck is o removed after five days. Tainsh (1944), who in sharp contrast to workers the Lister Institute and elsewhere does not favour resting periods for the s, reassembles at 100 to 120 days of age the bucks and does of the same litter ch were separated two weeks after weaning, removes does when pregnant, l returns them after they have weaned each litter.

In a survey of 352 litters selected at random from the records of several rs, Dewar (1945) found the average number born per litter to be 7·3, of ch 83 per cent. survived. Of the numbers surviving, the proportion of es to females was 10 to 9. The percentage of survivals varied with the nber of pregnancies, as is shown in Table 1.

TABLE 1

SIZES OF LITTERS

	1st litter.	2nd litter.	3rd litter.	4th litter.
of litters	100	100	98	54
rage number born ..	7·6	7·8	7·2	6·5
entage reared	74	88	86	83

Breeding continued satisfactorily from February to September, and 70 to per cent. of the does that were mated during this time became pregnant. tility declined during the winter months beginning in October and reaching inimum in December, when only 9 per cent. of mated does became pregnant. overy began in January and was complete by the end of February. The ediate cause of the decline appeared to be an anœstrus state in the females ; udy of the vaginal smears showed no regular periodicity, the diœstrus ar (leucocytes and mucus) persisting. Thus of 10 females examined from to 27th October, 1942, 2 came into œstrus four (normal) times, 3 three es, 4 once and 1 not at all. A pro-œstrus smear was often followed rapidly the metœstrus smear (leucocytes with a few squamous cells), the full rus stage being absent or transient. The males appeared to retain their ity. Such seasonal fluctuations in fertility are found to a greater or lesser ee in all colonies.

Breeding females should be inspected regularly, certainly not less than e weekly, for signs of pregnancy. These signs include a marked increase in ght, abdominal enlargement which Farris (1942, p. 1) says is evident at it the 13th day, and the palpable presence of embryos. Dewar (1945) ts out that embryos may be palpated earlier than the 13th day but that necessary abdominal manipulation is undesirable as a routine measure.

Pregnant does are removed to the individual breeding-cages and allowed ake their nest of suitable bedding material. In the average primipara in Wistar colony (Griffith & Farris, 1942) parturition begins at 22 days 14½ s (range, 22 days 10½ hours to 22 days 21 hours) after mating. (A range of 23 days is more generally accepted, and King (1913) has shown that the ation period may be extended by a week or more if the doe is carrying a large ber of young and is also suckling a previous litter.) A vaginal discharge is

evident usually from 1½ to 4 hours before the first of the young is born. "Th onset of parturition is characterized by rather regular extensions, with th interval shortening from 2 minutes to 15 seconds. During early labour, th female stretches while walking about the cage. With the approach of partur tion, the extensions become more severe. The female rests on her abdome and the hind limbs extend off the cage floor. Uterine ripples or contractur are frequently visible on the abdominal wall. . . . During the extension an contracture period the cervix dilates and the abdominal mass descends. . . The first fœtus is usually expelled by head presentation, and the others follo either by breech or head delivery. In most cases, the female licks the vul preceding the delivery. (Failure of the rat to lick the vulva seems to be corr lated with difficult delivery.) During delivery she stands in a semi-crouch position. . . . The female helps with the expulsion of the placenta by pulli it out with the mouth. She promptly eats the placenta, holding and working in her forepaws. . . . The mother licks the young rat and removes t amniotic sheath. The mother very seldom pays any attention to the you until the placenta has been eaten. . . . The mother finally assembles t young at the nest side. She then cleans herself and permits nursing. . . The length of time for birth of the primipara with litters averaging 11 young approximately 1½ hours (range 55 minutes through 3 hours 49 minutes)."

Apart from the interference necessary to weigh the newborn litter and select survivors, and for the changing of food and bedding, the nursing moth and her offspring should be disturbed as little as possible. (See under Sectio for handling of nursing does.) Figure 1 of the chapter by Farris illustra the distinguishing features between male and female newborn. "The male characterized by a larger genital papilla and a greater distance between t anus and the genital papilla. . . . Females lack nipples at birth but sh six pairs when they are from 8 to 15 days old. Occasionally supernumera nipples are present." (Farris, 1942, pp. 3–4.) Farris gives the average a genital distance in mm. of young albino rats as follows :

	Male.	Female.
Newborn	2·8	1·2
7 days..	5·2	2·7
14 days..	8·2	4·9
20 days..	12·0	7·0
42–50 days	21·0	13·0

The following data on age determination may conveniently be included this point. "The rat is born hairless, blind, with closed ears, undevelo limbs and a short tail" (Farris). According to Donaldson (1924) the hea always searching, with quick response to contact of olfactory and taste stim The approximate age of a rat may be determined from the information give Table 2, which is due to Donaldson.

As indicated on p. 123, there is a sharp division of opinion as to whethe not does should be rested between weaning one litter and being mated up for next. The majority view accords with that of the Lister Institute, wher long an interval as is compatible with the breeding schedule, i.e. even up month or so, is permitted. In many colonies the bucks also are rested for s periods.

The number of litters permitted per doe is usually restricted. At Lister Institute and many other laboratories not more than three litters

ually taken. Wood takes not more than four litters. Tainsh (1944) cards does after their sixth litter, or at any time earlier if they cease to roduce regularly and prolifically. He has found that fecundity increases adily up to the fifth litter and thereafter declines sharply. While the nopause in the doe does not usually occur until between 15 and 18 months age (Farris, 1942, p. 3), it is customary to cease to use both does and bucks routine breeding after they are from 10 to 12 months old. [Donaldson 24) assumes the life span of the albino rat to be about three years, although rris points out that where there is much humidity and a high temperature in mmer and extreme cold in winter, few individuals live for more than two rs. Slonaker (1912) has observed rats which lived for over 40 months in favourable climatic conditions in California.]

TABLE 2

DEVELOPMENT OF GROWING RAT

Before birth :	
Eyelids of fœtus fuse at	the 17th day
After birth :	Days
Ears open at	$2\frac{1}{2}$–$3\frac{1}{2}$
Incisors erupt at	8–10
Young find way to mother	9–11
Eyes open at	14–17
Hair obscures genitalia	16
Hair obscures nipples in female ..	16
1st molar erupts	19
2nd molar erupts	21
3rd molar erupts	35
Testes descend	40
Vagina opens	72
The menopause occurs at	450

The œstrus cycle. For certain types of work it may be necessary to practise vidual mating and to have as exact an idea as possible of the time of con- tion. For this a knowledge of the œstrus cycle is necessary. A detailed dy of the œstrus cycle in the rat was made by Long & Evans (1922), and iscussed with special reference to the Wistar colony by Nicholas (1942, 51–54), who may be quoted as follows :

" The œstrus cycle as described by Long and Evans extends over four days is divided into five periods. In most cases our experience has shown that cycle extends into and frequently through the fifth day. The separation hese five stages depends on the examination of the vaginal content and its elation with ovarian and uterine changes." These changes are summarized able 3.

The stage of œstrus may be diagnosed by activity measurements (Wang, 3 ; Farris, 1942, p. 2) or by a study of the changes in electropotential holas, 1942, p. 53), but the routine method adopted in most laboratories is microscopical examination of material taken from the vagina. Such erial, taken as illustrated in Fig. 31, gives a good indication of the stage he cycle.

Vaginal smears. There are several methods of taking a vaginal smear. rop of saline may be pipetted in and out of the vagina and then transferred slide. At the Lister Institute a metal spatula, moistened with normal

saline, is employed. Nicholas uses a small cotton-wool pledget, moisten with saline and inserted either with forceps or with the aid of a tampon ma from a toothpick. Material obtained by such methods is transferred to microscope slide for examination. At the Dunn Nutritional Laborator Cambridge, the smear is taken with the aid of a shaped glass rod which examined directly under the microscope. For routine examinations of smea stains are usually omitted. Where staining is desired, Nicholas recommen Wright's stain for speed and convenience.

TABLE 3

INDICATIONS IN THE ŒSTRUS CYCLE

Stage.	Superficial genitalia.	Vaginal smear.	Uterus.	Ovary.	Length.	Remark
I	Lips slightly swollen. Vagina dry.	Epithelial cells only.	Increased distension fluid.	Ovarian growth Follicle enlargement.	Ca. 12 hrs.	Heat m occur.
II	Lips swollen. Vagina dry.	Cornified cells only.	Maximum distension early. Regression.	Large follicles. Maturation of egg.	Ca. 12 hrs.	Heat. Copulate
III	Lips still swollen, regressing. Cheesy mass in vagina.	Cornified cells only.	Epithelium degenerating.	Ovulation	Ca. 15–18 hrs.	Not in he
IV	No swelling. Mucosa moist.	Cornified cells and leukocytes.	Begins regeneration.	Eggs in oviduct.	Ca. 6 hrs.	
V	No swelling. Mucosa moist.	Leukocytes and epithelial cells.	Epithelium regenerated.	Corpora lutea formed.	Ca. 57–60 hrs.	Œstrus.

Copulation may occur in late stage I, in stage II and occasionally in early stage III. Ovula may occur in late stage II and later. Copulation time usually precedes the ovulation, the li so far found being 6 to 30 hours. The copulation plug forms 3 to 8 hours after copulation ar not by itself an adequate criterion of timing.

Both males and females become sexually mature at 50 to 60 days of (Long & Evans, 1922). These authors state that the vagina opens at ab 72 days (range 34 to 109 days) and that ovulation occurs at 77 days (rang to 147 days). Farris (1942), however, quotes some recent observations w Wistar rats indicating that in about 60 per cent. of does the vagina open 35 to 50 days (range 15 to 67 days) and that in 85 per cent. of bucks the te descend at 18 to 31 days (range 15 to 51 days).

Vigorous Wistar albino males attempt copulation 15 to 70 times in 1 20 minutes, with one or two ejaculations, provided the female is in active h The female exhibits a typical lordosis during copulation (Griffith & Fa 1942). As noted above, a vaginal plug forms from 3 to 8 hours after copulat

Diagnosis of male fertility. For a method applicable to the rat, see Chapt p. 99.

[*Photo. A. W. Davies*

31.—Method of taking Vaginal Smear adopted at the Dunn Nutritional Laboratory, University of Cambridge and the Medical Research Council.

Note method of holding rat.

5. Handling and Marking

The domesticated strains of *R. norvegicus* are docile and healthy ; normally-animals which bite or show other signs of vice indicate faulty management. imals which develop the propensity to bite despite careful handling and per nourishment are suffering from some inherited or acquired disease and uld be eliminated from the stock colony.) Great emphasis must, however, aid upon gentle and considerate handling. As Hartwell (1944) suggests, should be so friendly towards the rats that they come to the front of the when one approaches. Rats are not normally lifted by the tail, as are , but are picked up gently with the palm of the hand over the back and the ers supporting the underside of the body. By rotating the wrist one can the rat quite easily with its ventral aspect exposed, and in this position ng, examination of various parts of the body, and the taking of vaginal ars are easily carried out. Gentle restraint of head and limbs may be ted with the fingers.

For *untamed* rats (see also appendix to this chapter), Griffith (1942, p. 18) ests a similar method, but with the thumb and index finger folding the nal's forelegs under its chin. "The animal cannot get its chin down to bite, the procedure, done correctly, causes little pressure on the trachea. Do squeeze the body. If the trachea is compressed the animal may have ratory difficulty later. Workman's gloves are worn to prevent scratches

from the nails of the hind feet. . . . If the rat is difficult to pick up it may grasped by the tail, whereupon it will pull away and may easily be picked while straining." Another method of restraining an untamed rat is to hold by the end of the tail with one hand and by the loose skin over the neck a withers with the other. Exceptional circumstances apart, however, untam rats should not be employed for routine laboratory work.]

Where nursing does are concerned Hartwell writes: "Never take a doe her young. Talk to her and she will come to you. If you take her forcib she *may* bite and will eat her young. Make a fuss of the mother by stroki and talking, so that she is not jealous of her young and will allow you to han them. With such treatment a litter can be removed and weighed under hours old."

Rats object strongly to noise (Donaldson, 1924; Hartwell, 1944; a others). Recent research on the production of audiogenic seizures in r (see Farris & Yeakel, 1943) bears out their marked susceptibility to noi which should therefore be avoided so far as possible in routine management.

Marking. Of the various methods of marking (see pp. 39–40), tattooi the ears and marking the ears with V-shaped snips are probably the m satisfactory. Dewar (1945) points out that snipping of the ears should be d in the membranous part away from the blood-vessels and when the animals young, preferably at weaning. A small, sharp pair of scissors should be u and the cut made radially towards the centre of the ear and taken no furt than the smooth, outer part of the pinna; little pain or bleeding then resu If much bleeding is avoided the edges of a single cut rarely rejoin, and remo of V-shaped snips is therefore unnecessary.

With the piebald or hooded strains, it is usually possible to distingu clearly the individual members of a litter, or the animals in a given experim by noting carefully the distribution of the black or chocolate markings, especi when sex also is taken into account. This method is adopted at the Li Institute, and has worked satisfactorily for years.

6. Anæsthesia and Euthanasia

Anæsthesia. Griffith (1942, pp. 19–22) describes methods of open eth zation, intratracheal ether anæsthesia, nembutalization and anæsthesia w evipan sodium, of which he found young rats were intolerant. He records t chloroform was most unsuccessful, and had to be abandoned.

Two well-tried methods will be described here.

Open ether. The successful way in which this method is practised at Dunn Nutritional Laboratory (by Dr. E. Kodicek) is as follows. The ra tied to the operating table and a beaker with cotton-wool soaked in ethe placed to the head of the animal. Care is taken not to touch the nose of animal with the cotton-wool, as this may precipitate a sudden fatal sh When complete anæsthesia is reached, the beaker is removed and etheriza is applied only when the rat shows signs of coming out of the anæsthe Respiration and corneal reflexes are continuously tested. The initial ph of the anæsthesia may start with violent convulsions of the animal, especi when the rat was excitable previous to the operation. When—quite rarel a primary shock appears, artificial respiration has to be applied. The recov from the anæsthesia is rapid and within 5 to 10 minutes the rats bel normally.

Tribromethanol (Avertin) anæsthesia. Brownlee (1945) generally adopts for small-animal work. Its features are smooth induction and recovery h surgical anæsthesia of some 15 minutes. It is especially suitable for ophysectomy in rats and mice, and is valuable also for operations on nea-pigs. The material, as supplied by Burroughs Wellcome & Co., is in tion in amylene hydrate, and is made up freshly as a 2·5-per-cent. solution aline at 60° C. The following scheme of intraperitoneal dosage obtains for colony with which Brownlee works but, as he points out, some variation n colony to colony is to be expected.

	Tribromethanol. ($2\frac{1}{2}$-per-cent. solution.)
Rats.	
30–60 g.	0·6 ml/100 g.
60–100 g.	0·7 ml/100 g.
above 100 g.	0·8 ml/100 g.
Mice.	
14–22 g.	0·5 ml/100 g.
Guinea-Pigs.	
250–400 g.	0·8 ml/100 g.
400 g. and over	1·0 ml/100 g.

Euthanasia. Most laboratories destroy rats by placing them in a closed nber containing ether or chloroform vapour, or coal gas led in from a h supply by means of pressure tubing. The efficacy of these methods is properly understood and in many cases marked struggling (whether con- is or otherwise) occurs. Overdosage with barbiturates and other narcotics most certainly painless but usually regarded as too expensive for routine Davidson (1944) practises decapitation with a single blow of a heavy o cleaver. Death is said to be instantaneous and painless and the method ted to be excellent for the purpose of obtaining tissues in as fresh condition ossible.

he subject of euthanasia in rats and other small laboratory animals ires much further study. The matter is not only of humanitarian impor- e but is also of great technical significance when histological or cytological inations are to be made. The personal communications of Baker (1945) apter 2, p. 44, and of Harper (1944) in Chapter 4, p. 80, should be read is connection.

7. Diseases of the Laboratory Rat

ver since the early days of preventive medicine in man and animals, s been apparent that the wild rat plays an important role in the mination of diseases caused by bacteria, viruses, metazoan parasites, In the course of investigations, the albino variety has become a widely and extremely convenient laboratory animal, susceptible as it is to ion with many of the causal agents of disease in man and animals. ddition to its use in pathological research, the laboratory rat is vourite experimental animal for physiological, pharmacological and ological research. It is, therefore, ever increasingly desirable that full exact knowledge of the natural diseases and parasites of the laboratory hould be collected in order that research workers may be enabled to ain the animals in good health and to recognize intercurrent infections

when they appear. Much valuable information and advice on this subject h
been collected and published in the invaluable textbook on *The Rat in Labor*
tory Investigation, by Griffith & Farris (1942); the chapters on "Protozoa
parasites," by Wenrich and on "Metazoan parasites," by Ratcliffe, conta
a great deal of information which can be supplemented by study of the pub
cations referred to in the bibliography. Similarly, the chapter on "Infectio
and non-specific diseases," by Ratcliffe, contains a great deal of useful inform
tion and advice; in the following notes, attention is drawn to recent observ
tions on infections of the nasopharynx, lungs and ears with *Streptobacill*
moniliformis and organisms of the pleuro-pneumonia group.

In the chapter on "Spontaneous diseases of laboratory rats" in the te
book, *The Rat in Laboratory Investigation* (1942), Ratcliffe remarks th
paratyphoid disease is the only specific infection at all common in laborato
rats, excepting, of course, certain conditions due to animal parasites. Howev
he mentions that about 75 per cent. of adult rats are affected with pulmona
disease and that middle ear disease and labyrinthitis are extremely common
certain colonies of rats. Recent research by Klieneberger and others has sho
that these conditions are associated with infection by organisms of the pleu
pneumonia-like group and it may well be that such infections are the common
found in laboratory rats.

Broncho-pneumonia of rats. According to Klieneberger & Steabl
(1937) a lung disease of laboratory rats has been well-known and recogni
as a clinical entity since the description of the condition in wild rats by Pe
& Macalister (1911) and Macalister & Brooks (1914). Other authors who h
described the disease are Tunnicliff (1916), Jones (1922), Meyer (1928), Nel
(1930 *et seq.*), Passey *et al.* (1936).

Tunnicliff (1916) described the appearance of filamentous structures
almost all of the diseased lungs which she examined and succeeded in isolat
a Streptothrix-like organism from over one-third of her cases; Jones (19
and Nelson (1930 *et seq.*) obtained cultures of organisms which were designa
B. actinoides muris and which were similar to the *B. actinoides* found
Theobald Smith (1918) in calf pneumonia.

Klieneberger & Steabben (1937) think that these cultures described
Tunnicliff, Jones and Nelson were really cultures of *Streptobacillus monilifor*
This organism, originally found in some cases of "rat-bite fever of man"
in "Haverhill fever" and later found causing "infective arthritis" of n
has been shown by Strangeways (1933) to be present in the nasopharyn
50 per cent. of apparently healthy wild and laboratory rats. Klienebe
(1935, 1936) claims that all cultures of *Streptobacillus moniliformis* are mixt
of the Streptobacillus in symbiosis with a very small pleuro-pneumonia
organism; she has succeeded in separating pure cultures of the latter organ
from the mixture and has classified a collection isolated from various pa
logical conditions of animals according to their morphology and cult
characters, designating them L_1, L_3, L_4, etc.

She noted that rats with lung lesions were more frequently carrier
Streptobacillus moniliformis than those with more or less normal lungs an
some instances has found the pleuro-pneumonia-like organism uncontamin
by the streptobacillus in the lung lesions. However, in severe cases,
organisms were present in the lung.

Pathology. Steabben (1937) states that "there are usually exter

nges in the lungs before any radical macroscopic alteration can be seen and difficult to judge from naked eye examination in the early stages whether a is or is not infected. Sometimes the pleural surface shows small petechial norrhages, sometimes it is covered with darkish pin-point gelatinous spots the major bronchiectatic lesions are developed in those parts of the lung t expanded in ordinary use ; these are fairly regular in their distribution in the order in which they occur. The small lobe is usually the first to ent a characteristic change, i.e. to become a mass of grey translucent ules ; then follow the apices of both the upper lobes and, later, the lowest of the lower lobes. The right side appears usually to be affected before left. Sometimes all the lobes of one lung are macroscopically normal, e the other is so changed that it seems very unlikely that it can still be tioning ; indeed the chief symptom of the more advanced condition is the in which the rat breathes. Instead of the rapid and shallow breathing of e normal animals, the movement of the flanks in the later stages is deep laboured, though the rats do not seem to suffer any impairment of appetite rdinary movement while they are under observation, a period covering, at ngest, about 18 months."

teabben points out that this condition must be differentiated from a true monia which may occur in laboratory rats when subjected to some sudden ge in the temperature of their environment. This " true pneumonia " sily differentiated from the lung disease under discussion since (*a*) the monia is acute and rapidly fatal whereas the bronchiectasis characteristic e lung disease is chronic and never fatal ; (*b*) the affected parts of the lung rey and translucent or sometimes hæmorrhagic but do not show the grey lar distensions of the chronic condition ; (*c*) the histological picture is al of a clinical pneumonia in which the alveoli are filled with fibrin and ; (*d*) a heavy streptococcal infection is usually found on bacteriological ination, whereas in the chronic condition the tissues are, when tested by ary methods, often completely sterile.

hose interested in the subject should consult the communications pub- l by Klieneberger & Steabben (1937, 1940).

urulent Lymphadenitis and Polyarthritis. A " pyogenic filtrable agent " described by Woglom & Warren (1938*a*, *b*). It was later shown by eberger (1939) that the causal agent was not a virus but a pleuro- monia-like organism which she designated L_4, a strain of which she had ed previously from a swollen gland in a rat. In 1939 Collier, in Batavia, ibed a polyarthritis in wild rats from which he isolated a pleuro-pneumonia- rganism later shown by Klieneberger to be identical with L_4. Similarly cases of polyarthritis in laboratory rats in this country another strain solated by Findlay, MacKenzie, MacCallum & Klieneberger (1939) ; at t was named L_8 but Klieneberger has shown that it is identical with The disease of rats described by Rhodes & Van Rooyen (1939) was bly the same condition as the polyarthritis investigated by Collier and ay *et al.*

e communications mentioned should be consulted for descriptions of the l and post-mortem appearances of the infection ; in addition, there is teresting communication by Findlay and his co-workers (1940) on the tibility of these pleuro-pneumonia-like organisms *in vivo* and *in vitro* to alts and other chemicals. In view of the prevalence of such infections in

rats and mice, claims to have isolated pleuro-pneumonia-like organisms fr rheumatic fever and rheumatoid arthritis in man by techniques involving t use of rodents should be accepted with reserve.

Middle ear disease and Labyrinthitis. King (1939) has pointed out th infection and inflammation of the labyrinth of the inner ear is an extension the inflammatory process from the middle ear and that the number of anim which exhibit symptoms of labyrinthitis usually is a small fraction of th in which infection of the tympanic cavity occurs. However, recognition suppurative disease of the middle ear is possible only when labyrinthitis developed also, in which case characteristic signs appear. King (1939) descri these as follows : " The first indication of its presence is a slight tilting of head to one side. Subsequently the rat holds its head continually close to floor of the cage with the nose pointing away from the medial line. Lack co-ordination in muscular movements is shown by the fact that the individ is unable to run in a straight line but does so in a curve. It may stagger a runs and if it falls on one side has difficulty in regaining the upright positi When suspended by the tail, the body invariably rotates rapidly. Becaus this latter characteristic, rats with labyrinthitis are often called ' twisters.'

" At post-mortem examination, the extent of the disease may vary w the animal. When clinical signs have been evident, inflammatory chan will be found in the structures of the inner ear as well as in the tympanic cav From the inner ear, the disease may extend into the petrous bone and audit nerve. In one or both ears greyish-yellow pus of varying consistency wit the auditory apparatus is the most obvious sign of disease."

Nelson & Gowen (1930) found that the incidence of middle ear disease of labyrinthitis varies with the colony. In some colonies about 50 per c of the rats have shown infection of the tympanic cavity when exami *post mortem*. Usually young rats under 3 months are not infected but incidence rate rises rapidly after that age. About 4 per cent. of those infe in the middle ear develop labyrinthitis.

Etiology. Ratcliffe (1942) referring to the work of Nelson (1930) an Smith, Bethune & Wilson (1930) states that bacteriological studies have inconclusive ; a variety of organisms have been isolated, sometimes in culture, at other times in mixtures. Often the exudate has been found t sterile, i.e. when examined by the more or less ordinary methods. Ne (1930–32), however, noted a correlation between the incidence of middle disease and of broncho-pneumonia and isolated the organism which he ter *B. actinoides muris* (? *Streptobacillus moniliformis*) from both condition many cases.

Further bacteriological investigation of middle and inner ear disease u Klieneberger's technique or other suitable methods for the detection of pl pneumonia-like organisms would seem desirable.

Control. Two methods of control have been developed. King (1 selects young breeding rats for pairing and segregates them in quarters sep from the original colony. The males are autopsied at once after mating the females after their progeny have been weaned. Only those young bred from parents free from middle ear infection were saved ; by following procedure through seven generations, a strain free from the disease was e lished. Nelson & Gowen's method (1931) was to separate the young from mothers immediately after birth, before the placenta was removed and

n to be reared by foster mothers from a strain in which middle ear disease labyrinthitis was very rare or unknown.

Ratcliffe (1942) suggests that these methods of selective breeding should ısed also for the establishment of colonies free from lung disease. In view he findings of Findlay and his colleagues (1940), *viz.* that *Streptobacillus iliformis* and pleuro-pneumonia-like organisms are susceptible to gold salt arations, chemotherapy with such substances might prove useful.

See also Nelson (1940).—*Ed.*]

Paratyphoid of rats. Ratcliffe (1942) remarks that paratyphoid infections ıts are much more common than is generally realized and that the lesions aratyphoid have been attributed to experimental procedure on more than occasion in recent years. The following notes are abstracted from his ription of the signs and lesions of paratyphoid.

Symptoms and lesions may be acute with high mortality or relatively mild chronic, the latter form being more common. In the acute form, the ted rats lose weight rapidly, the ears become pale, the eyes show a choco- brown colour; there are brownish crusts about the nose and sometimes e is [illegible] rhœa. Examination of the blood reveals severe anæmia.

On *post-mortem* examination, the liver is found to be friable, pale and some- s mottled. Minute yellow-brown foci of necrosis may be seen scattered ughout the substance. The spleen is enlarged, the capsule tense, the pulp purple and irregularly mottled by small hæmorrhagic foci and yellow-grey of necrosis. The intestines may be injected and show hæmorrhages; the hoid tissue may be swollen and sometimes ulcerated. The lymph glands e thoracic cavity are usually swollen but the mesenteric lymph glands ly normal. In the lungs there are usually purpuric spots but pneumonia be coincident; within the heart muscle there are occasional irregular pale

n the chronic form of paratyphoid there may be no obvious signs of se, the animals breeding and growing quite satisfactorily although infected. ost-mortem examination, the lesions differ somewhat from those seen in the disease. The cæcum is enlarged and distended, its wall is thickened and tic and there are irregular elongated ulcers on the mucosa. The lymphoid of the ileum may be swollen but is usually not ulcerated. The mesenteric s are usually swollen and soft, often with cysts in them. The spleen may larged with dark red pulp. The liver is usually normal.

tiology. Several species of the Salmonella group of bacteria have been minated, the commonest being *S. enteritidis* and *S. aertrycke* (*S. typhi um*); these Salmonella are often found in wild rats which may be carriers. source of infection is the so-called "virus" preparations consisting of es of Salmonella mixed with some palatable substance and used as a n for wild rats. Thus laboratory rats may be infected by contact with ats or by contamination of their food with droppings of infected wild rats. bly the chief source of infection in rat colonies is the carrier animal which ot developed any signs of disease.

ntrol. Ratcliffe (1942) suggests that vaccination with heat-killed sus- ons of several types of Salmonella should be tried. The ideal method be to endeavour to build up a clean colony using the methods recom- ed for the establishment of a colony free from lung and ear disease. utions against contamination from wild rats and mice would have to be

taken and frequent bacteriological examinations carried out for the detection carriers.

Neoplasms. Any or all of the types of tumours which occur in otl mammals may occur in rats but the frequency with which any one type m appear varies with the colony and strain. Two studies of the spontaneo neoplasms occurring in rats have been published within recent years; one these studies, published in two parts by Bullock & Curtis (1930) and Curtis, Bullock & Dunning (1931) deals with 426 cases of tumours in the colony of the Institute of Cancer Research of Columbia University, U.S.A. the other study is by Ratcliffe (1939), dealing with 273 cases of tumours in rat colonies of the Wistar Institute of Anatomy and Biology, U.S.A. In Columbia University colony, the incidence was 1·53 per cent., in the Wis Institute colonies, 3·1 per cent.

The most common of all the tumours listed in these records are the fib epithelial growths, fibro-adenomas of the mammary glands of female ra these fibro-adenomas have been found to be associated with abnormalities the pituitary and ovaries. Further publications on this subject have b listed by Ratcliffe (1942), viz. Geschichter *et al.* (1934), Heiman (1934), Hein & Krehbiel (1936), Wolfe *et al.* (1938), Bryan *et al.* (1938).

Disease of the Heart, Blood-vessels and Kidney. In his review, Ratcl (1943) says that the best known of the spontaneous diseases of the heart a kidneys seem to be referable to alteration in the vascular system and that the best of his knowledge, only one detailed study of these conditions i colony of rats has been published, viz. that by Wilens & Sproul in 1938 266 female and 221 male rats of the Osborn-Mendel strain maintained at Department of Chemistry in Columbia University.

It is interesting to note that these observers found that in 75 per cent. of animals examined there were lung lesions of bronchiectasis and suppurati middle and inner ear disease was also common. The relationship between th suppurative lung and ear infections and the various heart, blood-vessel kidney conditions listed does not seem to have been investigated.

Those interested in the subject should consult the publications by Wi & Sproul (1938), Fox (1933) and Hueper (1935).

General instructions on the control of ecto- and endo-parasites are give Chapter 3.

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APPENDIX

WILD BROWN RATS

(*a*) Notes by H. V. Thompson, B.Sc.

(Bureau of Animal Population, University Museum, Oxford)

Accommodation. Wild brown rats have been kept in the laboratory 12-in. × 12-in. × 7½-in. rat cages and in 36-in. × 24-in. × 12-in. colony cage Provided with hay and a 2-lb. or 3-lb. tin in which to nest, most wild rats sett down to captivity after about a week and cease to hiss and shriek at the sigl of a human being. Some, however, do not eat in captivity but fret and soo die.

Feeding. They have been kept for months in good health fed solely on whc English wheat and tap water, and without litter.

Breeding. No attempt has been made to breed wild brown rats in captivi but some have been pregnant when caught and have reared litters in sma laboratory cages. The eyes of the young were found to open at 19 days (weig 20 g.) and two young separated from their mother at 28 days (30 g.) continue to live and increase in weight. Young rats weighing 24-g. and 18-g. recover from nests in ricks did not feed in captivity, although offered a variety of ha and soft foods, and died within three days.

Crossing with white rats. One male brown rat was mated with four fema white rats in a 36-in. × 24-in. × 12-in. colony cage in February 1943. Th lived together peacefully; all four females became pregnant and gave bir to 36 young between them in March. This F 1 generation bore a gene resemblance to wild brown rats but were slightly lighter in colour and some them had white patches on the ventral surface and a white tip to the ta They attained a greater weight than white rats (e.g. adult male 490 g. as again 350 g.). The F 2 generation consisted of the albino and wild types in abo equal numbers. Back cross of F 1 male to albino female produced offspring the following types: apparently normal albinos, which did not breed tru brown and black hooded, black, and wild, type brown.

Transport. During 1944–45 140 wild brown rats were sent by rail fro Northumberland to Oxford (300 miles). The best vehicle was a dustbin with wire-netting cover, packed with straw, brushwood and a liberal supply of w sausage rusk.

(*b*) Notes by Dennis Chitty, B.A.

(Bureau of Animal Population, University Museum, Oxford)

Outdoor cages and handling. Colonies of at least 20 rats can be successfu maintained in outdoor cages of wire-netting with floor space 10 ft. × 5 ft. a 5 ft. high. The roof and preferably the sides should be covered with roofi felt, otherwise the floors become very dirty when it rains. Straw should provided in a nest box, in fairly limited amounts, so that the rats can counted periodically without the necessity of first removing much of the stra A daily check on numbers is essential in carrying out experiments on food a water intake, in order to allow for births, deaths, and the fact that corpses a generally eaten. Also certain types of food are carried into the nest-box a stored.

An apparently satisfactory diet is wheat, cabbage and Oxford tap water, the latter provided in a large bottle and tube supported on its side in a frame. Rats live well for many months, put on weight and bring up litters, which in turn have bred under these conditions. Up to seven full-grown rats, besides the female and her young, have occupied the same nest box, internal dimensions 2 ft × 1 ft. × 1 ft. high.

Marking and weighing rats is fairly simple once the rats are in a sack. The best way of getting them there is to keep a small box with its entrance hole just outside the entrance to the nest box (which should be of rat-proof material so that it has only one entrance). When the lid is lifted off the nest box the animals run into the small box outside and can either be weighed in it all together or tipped into a sack and examined individually. In this case the box should have no bottom, or a removable one, so that the mouth of the sack can be placed under the box before the rats run in. The box and sack are raised so that the rats fall to the bottom of the sack, which is then placed on the floor and rolled down until a rat's back is exposed, when its tail is lifted up and the animal is sexed. Rats in a huddle are very docile and when handled endeavour only to push their heads further into the mass of bodies, so that it is unnecessary to wear gloves, though it is wiser to do so because of the danger of rat-bite fever. While the tail is still held with the left hand the fur is clipped with a pair of scissors. There are fifteen combinations of clipping at four standard spots, and with sex and size differences this is ample. These clippings must be renewed periodically.

Next, the animal is grasped—palm of the hand on its back and fingers round its thorax—and pulled out of the huddle. A tin must be ready for it the minute its head emerges and the rat will dart inside. The open end of the tin is then placed against an upright surface until the lid is put on. The last rat or two may be more restive, but can be controlled by pressure of the hand from outside the sack.

[*Note added in proof, January*, 1946. Mr. Chitty has been responsible for a system of record-keeping (see p. 122) which appears to have many advantages. Those interested should consult him at the above address.—*Ed.*]

CHAPTER 7

THE BLACK RAT

(*Rattus rattus*)

By the late PHYLLIS KELWAY
(*Arkholme, Almondbury, Huddersfield*)

1. ACCOMMODATION. 2. HANDLING. 3. NUTRITION. 4. BREEDING. 5. DISEASE. 6. GENERAL BEHAVIOUR.

[*Editorial Note.*—The late Miss Kelway completed the preliminary draft of the repo upon which this chapter was to have been based two days before her death after a lo illness. It has therefore been necessary to substitute her draft report for the chap originally planned.]

1. Accommodation

The first batch of rats arrived unexpectedly. As the only houses availab were constructed of wood and were therefore gnawable, I rapidly construct a house while the rats remained in their store cages. I built the house 1½-in. × 1-in. battens, working with ½-in. wire-netting on the inside. It w 3 ft. square, and the door was the entire area of one side, fixed with two hing to drop down. Two hooks and eyes were used to fasten it. I had intended t cage to hang on the wall of a wooden hut measuring 16 ft. × 8 ft., but in haste I did not think of measuring the width of the door, which unfortunat proved to be 2 ft. 6 in. As the 3-ft. cage obviously would not pass through t doorway, I had to fix up the rats temporarily in the case outside ; but I ma new cages, 2 ft. square, as quickly as possible. The behaviour of a black fem during the first day showed that if she gnawed at the netting in the same pl long enough she might gnaw a hole and escape into the open, whereas if a rats should get out of their cages within the hut I could catch them again.

This first batch was a family consisting of a black female and six you I made a nest-box out of a margarine box by fixing a lid and cutting a hole the side as a doorway. I filled it with hay and the mother found it after had spent an hour examining every inch of the cage for a way of escape. Af twenty-four hours the entire family accepted the cage and the nest-b growing less wild as they became used to me, but they never fed until d unless driven by hunger. As hunger always brought on a bout of gnawing learned never to let them run short of food. I dared not risk handling th rats while they were outside, for fear they would escape, but as soon as four 2-ft. cages were completed I shifted the lot by trapping them in ordin box wire rat-traps. I sexed them as I did so by managing to work the through a gap in the wires, holding on and examining the sexual orga "Tailing" them in this way required care, as the skin slips off like a glov you hold on to the tip. The grip must be as near the base of the tail as possib you can then hold on as hard as you wish, as the skin will not slip off even w the rat strains away. The litter consisted of four males and two females.

ter date I mated the dam to her son, and the sons to their sisters. As the lting young were all black, I used this stock as pure *R. r. rattus*.

There was room for four of the 2-ft. square cages to hang on one wall of the den hut. I hung them just under the roof about 3 ft. from the ground. s method of building cages of netting all over worked very well, as practically leaning was necessary, all the droppings falling through the wire floor. The had therefore the minimum of disturbance. I am sure this freedom from rference induced them to breed more freely than if I had been forced to clean r cages out regularly. On the floor of the hut beneath the cages I spread

FIG. 32.—BLACK RAT EATING.
(Copyright: Phyllis Kelway.)

lust with a generous sprinkling of Sanitas powder. I cleaned this away as ired, and the hut never smelt of rats.

n each cage I put a nest-box (or sometimes two). In order to give more e to the rats I cut two battens 1 ft. 1 in. in length for each cage, drove t 2-in. nails into each end with about 1 in. sticking out, then fixed the battens t inches from the floor by hooking them horizontally on to the netting walls. vn rats could reach the nest-box either by jumping, or by climbing up the s; but when young were present I made a ladder of a batten to lead to the hole, keeping it in place by a nail in one end which went through the wire- ng of the floor.

The nest-boxes were of wood, measuring 9 in. × 6 in. × 4 in. The roof

was hinged to make examination easy, and there was a pophole at each end fo the rats to enter either from the back or from the front. For bedding materi I used hay and straw.

For some months these four cages in the hut and the 3-ft. cage outside we all I had. Specimens of *Rattus rattus* and *R. r. alexandrinus* were sent to m at a later date, and an increase in the original family of *R. r. rattus* cause considerable congestion. For a time I ran colonies of mixed colours togeth (six adults in each house) but this was unsatisfactory from the mating point view, so I had blocks of wooden hutches made by a joiner. These had outsi

Fig. 33.—Young Black Rats Eating.
(Copyright: Phyllis Kelway.)

measurements of 4 ft. 6 in. high × 4 ft. wide × 2 ft. deep. Six compartme went to each block. The hutches were lined with asbestos sheeting where possible, but even so, parts of the wood framework were exposed inside, and rat could wait an hour for its food without putting in time at gnawing a available edge. I then had other houses made with $\frac{1}{4}$-in. netting on two si and on the door; the roof and back being of asbestos sheeting, and the fl of wood. As the wire-netting and asbestos were now all on the inside of framework there was nothing for the rats to gnaw, except the nest-boxes. this time most of the stock had been bred on the premises; these sho distinct signs of being domesticated and were in every way better beha than the original wild specimens.

2. Handling

Whenever I needed to examine or remove a rat my chief object was to d upsetting the subject's temper. When a rat started squeaking in fear, nen I received a bite, I considered I had failed. At all costs I tried to give rats confidence. This meant considerable time being spent upon them. so required patience. The time was not wasted, for in two instances pairs I had not had time to " tame " failed to breed.

Tailing " a wild black rat is not easy. You need a quick eye, a steady and firm yet speedy movement. If you catch hold of the tail too near ip you not only run the risk of skinning it but you invariably receive a bite. The black rat bites hard. It has two methods of biting : one, the nip, and two, the cruel bull-dog bite when it does not let go until its tail ched. When biting in this second way it fixes its dark eyes upon you while ms literally to sink its teeth into your bone. " Tailing " a wild specimen another disadvantage. Many individuals acquire a habit of turning ly round and round with an action that makes you think you are having a crew twisted between your fingers. You have the sensation that you will untarily let the rat go at any moment. A rat having these corkscrew s nearly always brings the matter to completion by flinging itself upward flict a severe bite upon the nearest part of your hand. I tried wearing er gloves, but gloves were either too thick or too thin, and I gave them up. of the bites I received turned septic ; and after a few months of trial and I never expected to be bitten.

also attempted the method adopted in laboratories where intractable os, or specimens of *rattus norvegicus* are being handled. With the hand the rat's back, I clasped my forefinger and thumb across the front legs th the chin, holding them firmly. It is said the rat cannot get its teeth far enough to bite when in this grip. This was not my experience. The rat is exceedingly quick and elusive in its movements, and it is difficult case to get the right hold. I managed to tame some individuals so that ld stroke their backs, but even with these, holding them in the hand diately set up a biting complex. Many would take food from my hand ; walked on to my hand and up my arm, but all were alarmed by any cal grasp upon their bodies.

he most peaceful way of examination was to coax the rat into a wire trap.

3. Nutrition

uring the first two years of war the rats were fed mainly on boiled potatoes, n a great variety of scraps brought by friends. The scraps consisted of bread, buns, meat and fish-skin. At one period the meat and fish were t. To give some form of balance to the diet I gave raw horse-meat once k. One female devoured her young when on this diet. I therefore gave oked instead of raw meat, and she reared subsequent litters successfully. rough help from Mr. Charles Elton I was able to get coupons from the ry of Food. I cashed these in various ways, but mostly in home-grown The rats were in better condition when fed on unmillable wheat and potatoes than on oats and potatoes. For variety the rats still had the old scraps. I gave the boiled potatoes whole to save labour and did not them but when I gave a surplus the rats carried whole potatoes to their

nest-boxes. Some boxes were quickly made wet by the potatoes, and on wet, the rats had no hesitation in using them as lavatories, although th were normally very clean in their habits. I learned to give only enou potatoes for the day; the rats did not carry off the surplus grain but ate straight from the food dish.

In hot weather the rats drank a great deal and I tried various methods supplying water. S.D.I. lemonade bottles fixed in wooden supports like chic fountains were reliable, as they needed filling only once a week. Unfortunate I was in hospital for many weeks, and when I returned I found that the wood supports had been gnawed to skeletons. The rats must have been short food on some occasions for they had never gnawed them before. After this fixed metal pans on the wire-netting walls. These were easily filled by watering-can from outside without the door being opened.

With the exception of the litter eaten by the female as mentioned above lost no litters so long as water was supplied.

4. Breeding

Although I had no means of knowing the age of rats sent from the doc the young I bred in captivity mated readily from 12 weeks of age onwar Due to shortage of room the first litter was born when two male *rattus rat* were running with four female *R. r. rattus*. The males showed no tendency fight one another, or to interfere with the litter. The entire colony v peaceful, but no further litters were born until I had removed the six young four weeks.

In other cages I later ran one male with three females, or put them in pa Introducing strange males to two or more females often led to trouble. (cage housed three females (two blacks and one grey), but when I put in a g male one of the black females killed him immediately. I then put in two g males. These two were killed. I put in a *R. r. alexandrinus* but he dead on the following morning with bites about the head and neck. Th males were not attacked until they attempted to get into the nest-boxes. working the other way round, i.e. putting the females to the male or ma females would at first show fight, but only in a half-hearted manner and males showed the usual curious sex interest, but never fought. The best w therefore, of matching up a harem seemed to be to get the male established then to introduce the females. Once the harem was fixed, all went smoot even after young were born. Females with newly-born young were a touchy toward both inquisitive males and females, but temper never ran h enough to cause a death. It struck me that the females were always m possessive of " place " than the males were.

In the hutches where I kept pairs I at first put two nest-boxes so that male might have a flat of his own when young were born. No male made of this system until the growth of the young caused such congestion tha move was obviously desirable. And then the nest-boxes became interchan able for the entire family until the next litter was born. When I provided nest-box only the male revealed himself as an excellent father, carrying f into the nest in such quantity that for the first two days the female seeme stop inside and eat what he brought in. The female black rat is a devo mother. On being disturbed she will shift her young to new quarters again

in. She has no orthodox way of carrying the young but grabs an arm or or the scruff of the neck without discrimination. Before moving house she a clear-cut plan and always prepares a new nest before shifting the young. The female on heat shows more inclination to run about. She cleans herself n and squeaks if she bumps into another rat at the pophole-door. In a e cage there is much chasing before copulation takes place. I was surprised nd that when two males were after the same female one of the males would up the pursuit very quickly, leaving the field clear for his rival. Male *norvegicus* will often bite one another in similar circumstances.

The usual period of gestation is 21 days. Two black females had young he 23rd day. Weight at birth was between 4 and 5 g., but as these weights from average litters of six and seven or eight, it may be that the weight of vidual young taken from litters of from ten to twelve may fall below 4 g. d not breed any litters above eight in number. The males seemed to grow faster than females, as will be seen from the following table of two young m a litter of six *rattus rattus* :

i 29 *July*, 1942.		♂ (g.)	♀ (g.)
July	29.. ..	5	4
	30.. ..	6	5
	31.. ..	7	6
Aug.	1.. ..	8	7
	2.. ..	9	8
	3.. ..	10	8
	4.. ..	11	9
	5.. ..	13	10
	6.. ..	14	11
	7.. ..	16	11
	8.. ..	18	12
	9.. ..	19	12
	10.. ..	20	13
	11.. ..	21	14
	12.. ..	22	15
	13.. ..	23	16
	14.. ..	23	17
	15.. ..	24	19
	16.. ..	25	20
	17.. ..	25	21
	18.. ..	26	23
	19.. ..	27	24
	20.. ..	28	26
	21.. ..	28	27
Aug.	22.. ..	29	28
	23.. ..	30	29
	24.. ..	31	30
	25.. ..	32	30
	26.. ..	33	31
	27.. ..	34	32
	28.. ..	36	33
	29.. ..	38	34
	30.. ..	40	36
Sept.	1.. ..	42	37
Oct.	1.. ..	120	108
Nov.	1.. ..	190	170
Dec.	1.. ..	201	190
1943.			
Jan.	1.. ..	208	196
Feb.	1.. ..	202	193
March	1.. ..	200	190
April	1.. ..	204	193
May	1.. ..	209	196
June	1.. ..	208	197
July	1.. ..	210	197
Aug.	1.. ..	211	197
♀ Died 10 Feb. 1945			180
♂ Died 17 Mar., 1945		198	

oth male and female here were well-grown and perhaps on the large side. mallest in this litter, a female, weighed 151 g. on August 1, 1944 ; the only male weighed 198 g. on that date. The litter consisted of two males and emales.

litters resulting from hybrids of the cross *rattus rattus* ♂ × *R. r. rattus* ♀ acks were consistently lighter than the greys, irrespective of sex, although

nere a black ♀ and black ♂ were represented the male was usually 1 g. heav than the ♀ at birth ; or in cases where it was the same weight at birth, it ma up later by a more rapid increase. I noted the same sex difference in t greys.

Average weights in grams at 12 months of specimens bred and reared confinement were as follows :

Rattus rattus.	*Rattus rattus rattus.*	*Rattus rattus alexandrin*
♂ 203 ♀ 193	♂ 209, ♀ 198	♂ 195, ♀ 181

The young were the usual naked and blind young of rats. The eyes oper from 14 to 16 days of age. I weaned one litter at 19 days, feeding them bread and milk, which the young managed to lick up for themselves. Th needed a lot of attention and the earliest safe weaning age appears to be 21 da The mother will feed the young up to 28 to 32 days if required, but as fr litters are frequently born when the growing young are 21 days old, the f litter is then weaned. It is difficult to ascertain for certain whether three-week youngsters are entirely weaned when the two litters are in such cl confinement. The female shows unexpected patience with her double fam and manages to suckle and rear the younger lot while the older are runn recklessly in and out of the same nest-box. The female black rat is an amia mother, suffering much from both the male and the young ; I have seld noticed a mother chasing away the first litter, and when she has done so, it been a mix-up over the food problem—a bit of jealousy or greed that mi happen at any time irrespective of mothers and children.

These litters consisted of blacks and greys. I could tell the blacks from greys at 12 days by the darker tinge of skin and growing hair of the bla compared with the pinker skins and lighter hair of the greys.

No young were lost during the nursing period, with the single excep when the female devoured the entire litter. The young went ahead be when kept in single families than when housed in colonies of the same The toughest were more inclined to get the pick of the food when sev families were housed together, and individuals would fall far below t companions in weight and in size.

Reproduction rate of females. Where pairs were housed together the fem frequently mated a day or so after the birth of a litter, but in colonies of and 2 ♂ or 6 ♀ and 1 ♂ this occurred once only. The system of keeping male always with the female in the comparatively small space of 24 in. × 1 × 22 in. approximately (inside measurements) was conducive to a much hi rate of reproduction than the colony system. In colonies the females (espec marked individuals) failed to come on heat as regularly as those kept consta with a single male ; also certain females appeared to be sterile, as they did breed at all.

My rats bred during the spring, summer, and early autumn only, and n winter. As no heat was available and the temperature in the hut du normal weather was seldom more than 5° F. above the outside temperatu did not expect winter breeding.

The menopause seems to occur between the ages of 14 and 17 mo The age of 480 days below was the minimum age for the last litter t born, and 512 days was the maximum. Most females fell midway betwee two.

TABLE SHOWING REPRODUCTION OF 3 ♀ FROM BIRTH TO DEATH. LIKE BEING MATED TO LIKE.

	Age of ♀ at which litter was born in days.	Total number in litter.	Males.	Females.
Rattus rattus	93	8	6	2
Born March 1, 1942; died	121	8	4	4
Nov. 5, 1944.)	160	6	5	1
	200	8	6	2
	361	5	3	2
	422	5	2	3
	503	4	2	2
	Young were all grey.			
Rattus rattus rattus	103	6	3	3
Born June 5, 1942; died	147	8	5	3
March 3, 1945.)	169	6	2	4
	203	7	3	4
	250	5	3	2
	292	5	3	2
	353	5	2	3
	480	6	4	2
	Young were all black.			
Rattus rattus alexandrinus ..	111	8	4	4
Born April 17, 1942; died	194	7	5	2
March 6, 1945.)	337	6	4	2
	399	6	3	3
	456	4	3	1
	512	6	4	2
	Young were all grey.			

I tried keeping *R. r. rattus* and *R. norvegicus* together. Where the two ies were not placed together with some care *R. norvegicus* promptly killed . *rattus*. By placing *rattus* in the 3-ft. cage first so that it was established, by providing three nest-boxes, and introducing *norvegicus* after a week or I managed to persuade them to live together in peace. In every case the eventually shared the same nest-box, although they kept strictly to rate nest-boxes for about a month. I put the following adults together :

(1) Tame white ♂ with *R. r. rattus* ♀
(2) Tame white ♀ with *R. r. rattus* ♂
(3) *R. rattus* ♀ with *R. norvegicus* ♂
(4) *R. rattus* ♂ with *R. norvegicus* ♀

} For six months of spring and summer.

(5) *R. r. alexandrinus* ♀ with *R. norvegicus* ♂ for 12 months.
(6) *R. r. alexandrinus* ♂ (born from No. 3 mating and put together at 12 weeks) with *R. norvegicus* ♀ (bred in captivity, age 13 weeks) for life.

R. r. alexandrinus died first.

No mating occurred in any of these experiments. After the first slight on introduction no fights took place, as far as I know. The two species ed a tendency to feed apart, *norvegicus* naturally coming down from the box first and *rattus* showing a greater timidity.

(*a*) On mating a black ♀ from No. 2 mating to grey ♂ from No. 1 mating I ned 6 young, all black.

(2*a*) On mating black ♂ from No. 2 to grey ♀ from No. 3, 7 young were bor all black.

(3*a*) On mating grey ♂ from No. 1 to grey ♀ from No. 3, 5 young were bor 4 greys and 1 black.

(4*a*) On mating black ♂ from (1*a*) to black ♀ from (2*a*), 5 young were bor 4 blacks and 1 grey.

I killed off most of the young after weaning, but no young were lost duri the nursing period.

The young are as easily tamed as the young of *R. norvegicus* and show t same high intelligence. The mother resents interference, especially if t young are removed from the nest-box. To examine them I took out the wh nest-box. The mother accepted this method more calmly than if I poked in the house, as she seemed to wish to attack and yet to fear to do so.

Age limit. Owing to shortage of space I was unable to keep many ra until they died from old age. I kept one male for four years and one mont but he was decrepit at the end and his hair was loose and staring. The ordina life span seems to be $2\frac{1}{2}$ to 3 years but it must be remembered that of th that died within this period, three females in No. 1, 2 and 3 were hard press to produce as many young as possible, and no help was given them in th rearing. The longest lived were always the males.

5. Disease

The rats were all very hardy, the temperatures in their houses being lit above that outside, but the nest-boxes were warmly filled with hay. As as I know there were no cases of pneumonia. I rarely found a flea, but in c case an old rat was troubled with lice, but he was living by himself and ot rats were not affected. This absence of fleas and lice may have been due the open nature of the cages and the ventilation, for the nest-boxes were washed often, although each time one was cleaned of litter it was freely dus with Sanitas powder.

It is curious that the species was not affected by mite in the ears, fo had tame white rats at the same time that sometimes had very bad ears a had to be treated with sulphur ointment. The same apparent immunity not apply to *R. norvegicus*, even when these specimens were caught from wild. Wild *norvegicus* became infected when housed with a tame white i bad state ; but so far as I know no individual of either *R. rattus*, *R. r. ra* or *R. r. alexandrinus* ever had scaly ears due to this mite.

6. General Behaviour

The black rat does not become so docile as *norvegicus* under similar co tions. I had no time for much handling of individuals, all handling b necessary to weighing, sexing, examination, etc., but in temperament black rat seems to be more highly strung and less willing to be domestica It has a more independent nature and will (when first caught) suffer real hu and discomfort rather than take risks. In every way it is more agile an never lazy. *Norvegicus* can show a certain indolence in captivity tha absent in the black rat. Less pushing in every way than *norvegicus*, the b rat is, I think, more cleanly in habits and more fastidious in every act

confinement it spends much time in washing itself, and the dam is scrupulously clean in the nursery as long as she is allowed to be. The animal seems to less of a scavenger than *norvegicus*. I found that, although it would devour meat readily it would leave " high " meat until forced by hunger to eat it, rain, bread or clean scraps were on the menu. *Norvegicus* would tuck into l in preference to the cleaner foods. Both species enjoy picking a bone, the black rat does not eat offensive material unless obliged by shortage of er food. The black rat is less pugnacious than *norvegicus*. It is not rrelsome with members of its own species ; so long as nursing mothers are unmolested few disagreements take place. Even rival males seem to w when to abandon the quest without blood being drawn. I once housed males and a single female together. The males paid no attention to one ther, even when the female came on heat. Yet a group of females seem to e considerable objection to a strange male coming suddenly into their st.

The courage of *norvegicus* when in a tight corner appears to be greater than of the black rat. *Norvegicus* will bare its teeth, squeal and even launch to ck, but the black rat is inclined to squeal, shiver from fear and duck its d. Whenever a black rat got loose temporarily in the wooden hut it always le its way upward at the first opportunity, climbing the walls at the corners hiding beneath the eaves. It differed in this way from *norvegicus*, which ys went to earth behind or into the nearest available box on the floor. pite of the more excitable temperament of the black rat, it proved the gentler ies under all circumstances.

CHAPTER 8

THE MOUSE

The Maintenance and Handling of Mice

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1. Accommodation : the mousery. 2. Nutrition. 3. Breeding. 4. Handling. 5. Anæsthesia and euthanasia. 6. Some observations on the diseases of mice (by P. A. Gorer). 7. A note on the training of assistants.

1. Accommodation : the Mousery

Anyone visiting a number of institutes in the hope of being able to desi an ideal mousery might easily be bewildered by the variety of methods he sa It is clearly impossible to suggest any arrangement that will cover all requi ments ; thus the needs of somebody who wants large numbers of mice standard weight for biological assay are naturally different from those of geneticist. However, there are certain principles that must be observed by who wish to maintain healthy stocks. Assuming the stock to be healthy start with, the mice must be given an adequate diet, kept at an appropria temperature and protected from infections brought in from outside. [Gene notes on the construction of the animal laboratory are given in Chapter which should be read in conjunction with the present section.—*Ed.*]

The position and size of the mousery are usually determined by archit tural and other factors which need not be considered here. The optimum s of a mouse room is difficult to state. A room 12 ft. long would convenien take ten double cages, such as are in use at Guy's Hospital, on each side. It quite easy to work with three shelves on each side ; this allows 60 cages in t room. If each cage contained 20 mice (10 in each compartment) this wo give 1,200 for the room. In practice one seldom keeps the cages as crowd as this, and if breeding is being carried out the number of mice per cage much less. The breeding room at the Chester Beatty Institute measu 20 ft. × 18 ft. and accommodates 580 cages of the type shown in Figs. and 35.

Those who are setting out to establish a mouse colony will do well to b in mind that, as their problems grow, so will their demand for mice and t space to accommodate them. In general, several small, separate rooms preferable to a single large one. If all the mice are kept in one room and epidemic breaks out, the likelihood is that all will be infected, whereas if t mice are kept in a number of separate rooms it should be possible to con the infection to a single room. Those who use pure lines for experimen

ork have an additional incentive for using more than one room. A number of fferent pure lines are albinos and there is less danger of their becoming mixed they are bred in different rooms. It is, of course, important that each room ould be wide enough to work in comfortably.

FIG. 34.—A CORNER OF THE MOUSE-BREEDING ROOM AT THE CHESTER BEATTY RESEARCH INSTITUTE.

e the attachment to the cold-water tap for filling the water bottles. The heavy wooden structures and black-out curtain are temporary war-time necessities.

In addition to the housing quarters, a cleaning room will be required with ater supply, a large tank for washing the cages and, if possible, a large ilizer in which the cages may be disinfected. A room for the storage and paration of food is also needed.

When designing the mousery it should be borne in mind that the mouse

rooms and feeding-stuffs must be securely protected from wild rodents. Sever epidemics have been traced to wild mice. In one instance they ran over tl cages with the result that their urine and fæces infected the mice inside. In second case wild mice infected the sacks containing the feeding-stuffs. In third case they actually got inside defective wooden cages. As an addition protection against the introduction of infection by wild mice it is well to sto the food in metal bins rather than in sacks.

FIG. 35.—THE MOUSE-BREEDING ROOM ILLUSTRATED IN FIG. 34, SHOWING IN DETAIL THE METAL FRAMEWORK FOR HOLDING THE MOUSE CAGES.

In the construction of the mousery, woodwork should be avoided as far possible. Woodwork harbours bugs, is absorbent and has other objectional features. The walls and floor should be of non-absorbent cement or mater with similar qualities. The shelves on which the cages rest should be entire of metal, and in all situations corners should be avoided, all edges should rounded and all passage-ways for pipes through the floors or walls should made rodent-proof. The floor should be provided with a drain so that hosi is facilitated.

It is advantageous to have swinging doors so as to avoid the noise of amming and also to allow easy exit or entry when hands are full.

Temperature-regulation and ventilation. Mice breed best at a temperature hich does not deviate too much from 70° F. Below 60° F. one is liable to se numbers of young mice [This may not be true of all strains.—*Ed.*] and dden high temperatures seem specially bad for old mice. There can be little ubt that a cold and draughty mouse room is liable to increase the incidence respiratory infections. Doubtless the best method of providing the necessary ntilation with maintenance of heat and avoidance of draughts would be by -conditioning with the aid of pipes and fans. A less satisfactory but more sily available method is provided by a fanlight in the roof rising 3 or 4 ft. ove the roof-level, with glass panels and windows which can be opened or sed according to needs. A fan is a useful accessory for speeding the exchange air when necessary. Whatever system of ventilation is employed it is tainly desirable to exclude the entry of flies so far as possible. Although are not aware that any epidemics have been proved to have been started by s, the latter are known to be capable of spreading bacteria that are very ngerous to mice, and this should be borne in mind when considering ntilation.

Thermostatic control of the heating arrangements may be regarded as very irable, and for this purpose good insulation is of great importance.

Lighting. The rooms must be well lit and free from dark shadows, so that technicians can easily keep the mice under observation. Bad light obscures corners, and these require particular attention. Dead mice are apt to lie the corners or to be interred in the bedding. The artificial lighting should hung high enough to enable work to be done in any part of the room with ed, comfort and unimpeded vision.

Equipment. Each mouse room should be provided with a table or trolley which cages can be placed for inspection, a laboratory stool, and a sink with and cold water and a draining-board. It will be found convenient to have orizontal tube fitted to the cold tap and provided with two or more grease- nozzles as shown in Fig. 34. This fitting facilitates the filling of water- tles.

Racks and cages. The racks for the support of the cages should be arranged old the boxes safely, and suitably spaced to enable the easy removal of any e for inspection. The arrangement in use at the Chester Beatty Institute hown in Fig. 36. The main stanchions are six inches away from the wall. ch shelf consists of three metal bars, two at the same level to support the e and one slightly higher at the rear to prevent the box being pushed off he back. The lower shelves are one foot from the ground.

The cages may be made of wood, glass or metal (galvanized iron or zinc). od is popular and is cheap, but both of us have experienced trouble with -bugs in using wooden cages, whilst with glass or metal these do not occur. Chapters 2 and 3 for emphasis upon metal cages.—*Ed.*] Some workers a cage that has wooden sides and a metal bottom, the side pieces having s into which the bottom and end pieces slide. The whole is easily dis- nted and may, therefore, be thoroughly cleaned. We have had no personal erience with glass but it is used in a number of excellent breeding establish- ts. Goldfish bowls or accumulator jars have been used, the covers being le of fine perforated zinc with a hole for a drinking-bottle. They have the

advantage that inspection of the mice is facilitated and the jars may be easil cleaned and sterilized.

There are numerous types of metal cage on the market. It may be foun more convenient to use a deep cage than a shallow one; young mice jum about a great deal and can easily get out of a shallow cage when it is opene At Guy's Hospital we use cages 6 in. deep and a greater depth would not b disadvantageous. The tops should consist of perforated zinc or wire-mesh tha is fine enough to exclude flies b allows inspection of the mice witho the cage being opened. Provided t top is fly-proof it may be fitted on any number of ways. Whatever ty of fastening is selected, it should sufficiently strong to stop the mi from pushing the cage open and withstand wear and tear.

Fig. 36.—Framework and large Cages used for the Stock Mice.

(Chester Beatty Research Institute.)

At Guy's Hospital we use galv nized-iron cages with a partition do the middle on to which the fly-pro lids are hinged, and these are he down by clips on the outside. T whole cage measures 12 in. × 12 in plan and is 6 in. deep. Each ha cage has a rack to hold a card on whi particulars may be written. One m keep ten adult mice on each side such a cage. The lid has a h through which a drinking-tube m pass. The only drawback to t design seems to be that the clips the side are liable to break. T could probably be overcome witho much difficulty.

At the Chester Beatty Institu metal boxes are used (Fig. 34). Th measure 4 in. × $6\frac{1}{2}$ in. × $9\frac{1}{2}$ in. a the lids are perforated but fly-pro In each lid is an aperture for the spo of the water-bottle, and on the fro of each box is an attachment holding a small card on which written the identification numbers the inhabitants. Each of these bo conveniently accommodates five mice; the boxes economize space, all ready access for feeding and inspection, and are easily cleaned. For st mice larger boxes of the same type are used. These measure 4 in. × 14 × 29 in. and will provide temporary accommodation for fifty mice (Fig. 3

The cages should be changed at least once a week; after the soiled bedd has been scraped loose and tipped into a large-sized sanitary dust-bin kept the purpose, the cages should be well scrubbed in soap and water. Alter

ely they may be immersed in a large tank (e.g. 60 in. × 28 in. × 21 in.) ntaining a warm 2·5-per-cent. solution of lysol and then put in a steam rilizer at 100° C. for an hour. At Guy's we occasionally have a cage auto- ved at 15 lb. pressure for 30 minutes as an emergency measure. After ng cleaned the cages should be well dried before fresh bedding, etc., are in. Adequate cleaning and changing of the cages are of the utmost portance for the maintenance of healthy mice. About the only occasion on ich this procedure should be relaxed is if a mouse is actually in parturition f the onset of parturition is suspected, in which case the animal may be left ts cage for a second week. Because of the numbers in use it may be impos- e to sterilize all the boxes every week. In such circumstances as many uld be passed through the sterilizer as the existing conditions permit.

The soiled bedding contained in the dust-bin is best disposed of by burning, a special destructor for this purpose, and for the disposal of the remains of d mice, is a useful adjunct to the mousery.

Bedding. Clean, preferably sterilized, sawdust provides satisfactory ding. For breeding mice it is usual in some institutes to provide nesting erial for which hay, straw or paper may be used, but provided the room is m this is not necessary ; indeed, some workers claim to have obtained ter results without it, since young mice are sometimes lost in it. At the ster Beatty Institute a little hay is given for nesting. [N.B.—Hay may be taminated by wild rodents.—*Ed.*]

2. Nutrition

Although everybody of experience will agree on the importance of an quate diet, practically every laboratory uses a different one. Nowadays a nber of people are beginning to use pure lines of mice and there is no doubt certain of these are very fussy about their diet.

The specific nutritional requirements of the mouse have not received so detailed a y as those of the rat, for which see Chapter 6, p. 118. The qualitative exogenous irements for the vitamin B complex are probably somewhat different—for a recent y on the biotin and folic acid requirements see Nielsen, E., & Black, A. (1944), "Biotin folic acid deficiencies in the mouse," *J. Nutrit.*, **28**, 203–207. For a recent study on tamin E deficiency in the mouse," see Cerecedo, L. R., & Vinsen, L. J. (1944), *Fed.* ., **3**, 55. Two important papers on the feeding of the laboratory mouse and the effect et upon resistance to disease are those of Watson, M. (1937), "Studies of the influence et on resistance to infection," *J. Hyg., Camb.*, **37**, 396; and of Watson, M., Wilson, J., ppley, W. W. C. (1938), "The effect of diet on epidemics of mouse typhoid," *Ibid.*, 24–431. Woolley, D. W. (1945), has reported that mice require the component of in proteins, " strepogenin," that does not appear in acid hydrolysates, in his paper servations on the growth-stimulating action of certain proteins added to protein compounded with amino-acids," *J. biol. Chem.*, **159**, 753–754.—*Ed.*]

nadequacy of diet may be revealed in various ways. The litters may be ll and the animals stunted. Alternatively the animals may appear in llent condition and large litters may be born, most of which will be eaten eglected and left to die unfed. A less well recognized sign of unsuitable ing is a very high mortality about the time of weaning (about 4 weeks older animals being perfectly healthy. The young mice may have rhœa or may die without any obvious symptoms. Bacteria may be ted from the heart-blood of these sick animals but the infecting organism t always the same ; thus three mice from the same cage all gave different

cultures: one had a coliform organism, another *B. proteus*, whilst the thir had an organism that was probably a *Pasteurella* (Gorer, unpublished). A improved diet reduced fatalities very rapidly.

As a general rule the basis of the diet is some kind of biscuit. In Gorer view by far the best of these was Purina Fox Chow, an American produc unfortunately unobtainable at present. Until 1940 this formed the basis the diet, which, however, was supplemented by a seed mixture consisting two parts of wheat, oats, millet and rice to one each of dari and hemp see About ¼ pint was put in each cage every other day, the exact amount dependin on the number of mice in the cage. The seed mixture may probably be simpl fied without ill effect, and excellent resul were obtained with Fox Chow plus whea oats and rice. Latterly a rat biscuit mad by Messrs. Thorleys has been used in pla of Fox Chow. The results are not good. At present the diet consists Thorleys' rat biscuits *ad lib.*, and, once week, a mixture consisting of equal par of wheat, oats and rice with approx mately 5 per cent. dried yeast and 1 p cent. cod-liver oil.

FIG. 37.—CAGE DESIGNED BY BROWN-LEE FOR EXPERIMENTS INVOLVING CONTROLLED FOOD INTAKE BY MICE.

(Wellcome Physiological Research Laboratories.)

At the Chester Beatty Research Ins tute the following war-time diet is use Monday, oats; Tuesdays, alternate puppy biscuits and Thorleys' rat cak Wednesdays, alternately bread spre with Marmite and bread spread wi cod-liver oil; Thursdays, Millard Mar basal diet; Fridays, plain wholem bread; Saturdays, oats, to last for t days, no additional feed being supplied Sundays. Pregnant females are given small supplement of milk daily until t young are weaned. The amount of d food required per day for each mouse approximately 4 g.

Mice need ample to drink. Some peo give wet food, but drinking-bottles preferable. There are several patterns these, all of which are adequate. Th should be easy to clean and the stem should reach to within about 1·5 in. fr the floor of the cage. Often the stems are too short, with the result that recen weaned mice cannot reach them. An important aim in every nursery is keep the cages as dry as possible. The bottles in use fulfil this aim and a prevent contamination of the water by excreta. [For a note upon automa watering devices, see Chapter 2, p. 36.—*Ed.*]

The particular form of bottle chosen must be adapted to the cage in u At the Chester Beatty Institute we have employed for many years glass bu with nozzles of appropriate length. [These are made for us by Messrs. How Rawson & Co., Ltd., Devon Works, Farm Lane, Fulham, London, S.W.

e bottles for the small mouse-cages hold about 65 ml. of water and the nozzle 2 in. long. For the larger cages capable of holding 50 mice, the bottles have apacity of 165 ml. and the nozzles are $2\frac{1}{2}$ in. long. A special attachment to e cold-water supply which facilitates filling these bottles has been mentioned eady ; it is shown in Fig. 14 B.

It is confessed that there is room for much more controlled observation in ler to obtain an ideal diet for mice. Here it is necessary to stress that a diet ich seems excellent for ordinary mixed strains or even for certain pure lines y prove inadequate for another pure line. Probably the largest collection of e lines is maintained at the Roscoe Jackson Memorial Laboratory, Bar rbor, Maine, where Purina Dog Chow is used. [A design of cage which has ved of great value in controlled feeding experiments is illustrated, with t of the lid removed, in Fig. 37, and was designed by Dr. George Brownlee, he Wellcome Physiological Research Laboratory, Beckenham. The feed-cup elled " 5 " in the photograph) fits exactly into the upright cylinder shown t to it and is held in position by the nail. The mouse feeds from the cup r advancing along the horizontal cylinder extending from the cage. This ension is not sufficiently wide to enable the mouse to turn round. Hence tamination of the food through fæces is avoided, while scattering also is rcome. " The cage or mouse-box is a cube of $3\frac{1}{2}$-in. sides, is ventilated and ies a 25 ml. clear glass ampoule as water bath. A heavy lead (1/16 in.) lid t with glass is fitted. The lead is protected from the mice by a strip of zinc de. The side tube is 2 in. $\times$ $\frac{3}{4}$ in. closed by 20-gauge copper gauze dered). The food-pot is carried snugly in 1-in. bore brass tube and is ined by a $1\frac{1}{2}$-in. wire nail. The food-pot is a standard tube-cap in tin-plate aluminium, has a diameter overall of 15/16 in. and is $\frac{3}{4}$ in. deep. Its meter in 16ths of an inch describes its number and is known as aluminium tinplate) cap No. 15 and is supplied by Baker Melne Slopper & Co., Hadley et, Key Hill, Birmingham. The bore, length and height of the side arm critical. In this case, the side arm is inserted 1 in. above the sawdust le the cage, i.e. $1\frac{1}{8}$ in. from the floor. The figures given are suitable for mice o 22 g. weight."—*Ed.*]

[For reference to cube-feeding of mice, see p. 48.—*Ed.*]

3. Breeding

Mice reach puberty at from 6 to 8 weeks of age. Pregnancy lasts about lays, so the first litters are born when the mothers are from about 9 to 12 ks old.

Weaning may take place at 3 weeks, but 4 weeks is usually preferable. e people re-mate mice at weaning, whilst others prefer to wait until the ng mice are about 8 weeks old.

Pure lines have to be mated brother to sister and this often dictates the ber of females to be mated to a given male. One male to two females is e convenient but more females may be used if desired. Should an animal he requisite sex be unavailable, a back-cross to one or other parent may be without harm. For mixed strains, one male to five females is usually enient.

Pregnant females should be segregated unless there is only one female with male, when they may be kept together without harm. The mice may be d perfectly well even if there are several females pregnant, but the cage

quickly becomes overcrowded. Non-pregnant females will sometimes lacta and help to suckle the young.

If mice eat their young the reason is usually dietary. If they are well fe handling of the litter seems to make no difference. However, from time time one gets females who will eat every litter whatever one does. One cann judge the usefulness of a mouse by her first litter ; often this will be eaten a subsequent ones nursed and weaned without trouble. On no account shou mice be handled during parturition. Even if the doe is handled gently, labo may become obstructed or, if this is avoided, the litter is seldom raised a the female may subsequently prove infertile.

As a general rule mice will not breed after they are about a year old. It seldom worth remating them above this age even if their record to date h been excellent.

[For a method of diagnosing *male fertility*, applicable to mice, see Chapter p. 99.—*Ed.*]

Registration of births and identity marks. As soon as a litter is born t date with the number of young is entered on the mother's record. At the ti of weaning the sexes are determined and each mouse is given an identi number and receives a distinguishing ear mark. At the Chester Bea Institute ear snips are used. A more complicated system is illustrated Bittner (1941), the necessary mark being made in the ears with Spra Midget Toe Punch (designed for poultry).

The identity number of each individual mouse is entered on the recor is written on the box label, and accompanies the mouse throughout life, a when the latter dies the number is entered in the post-mortem book.

Each box is inspected daily when the food is given, and any sick mice reported. Dead mice are at once removed, a label bearing the mouse's ident number is tied to the hind leg, the abdomen and chest are slit open w scissors, injury to the viscera being avoided, and the mouse is then dropped i a jar containing 10 per cent. formalin in normal saline. In this way satisfact post-mortem examinations by naked eye and microscope can be obtained. T routine has been adopted at the Chester Beatty Institute because the dec position of mice after death may be very rapid. [For the post-mortem exami tion of mice in bacteriological experiments, see Chapter 2, p. 42.—*Ed.*]

4. Handling

Any rapid or rough movement is apt to alarm animals, in the managem of which the first principle is that all movements should be carried out slo gently and without sudden jerks. By far the commonest cause of being bit is fear of the animals and handling them roughly. The fact that a mous lively does not mean that it will bite. A little while ago one of us gave s rather lively young mice away. We were informed that they were q unmanageable and could not be held for injection ; previously they frequently been handled by a very junior technician who had been taugh hold them properly. Mice should be lifted by the tail ; sometimes they hang quite quietly, at other times they will curl round and crawl on to hand. It is when the latter happens that a lot of people think they are g to be bitten and consequently are bitten. The mouse should be allowe crawl in the palm of the hand ; the only trouble that is likely to occur is

may be reluctant to leave it. [Berenblum (1944), in the private communica-
n to which reference is made below, recommends holding mice as illustrated
Fig. 38.—*Ed.*] When it is desired to inject mice, they should be lifted by
e tail with one hand and put down on some rough surface, such as the wire-
uze lid of a cage, and with the free hand they should be grasped gently but
nly by the nape of the neck. It is important that a rough surface should
used, because then the mouse will pull away without turning its head. On
mooth surface it will slip and turn its head ; it may then see the second
nd coming and try to bite it. If the operator has an assistant, the latter
l hold the mouse with both hands ; however, the operator, having secured
neck between the thumb and index finger, may transfer the tail to the last
o fingers of the same hand,
ving the other hand free for
syringe. Mice get used to
ng handled and if repeated
ections are given they get
ier to handle on each occasion,
n though the needle must
t them a little.

Fig. 38.—Method of Holding Mouse recommended by Berenblum.

(Sir William Dunn School of Pathology, University of Oxford.)

Mice of various strains differ
temperament ; some squeak
the least provocation, whilst
ers only do so if obviously
t.

Male mice are liable to fight
ut together. If weaned be-
puberty and put together
y rarely fight amongst themselves even when mature, unless a strange
e is introduced, in which case a free-for-all may ensue. Mature males
usually fight for a day or two after being put together, after which they
ally stop, except that a short outbreak is likely to happen just after they
e been put into a clean cage.

Serious injuries as a result of fighting are rare. Occasionally one may meet
rogue " mouse who will bite all the other males in a cage and inflict serious
ry. The others never seem to retaliate and the rogue may be recognized
the fact that he is the only mouse uninjured. The bites are commonly
ined to the genital organs and to the back, which may be made completely
. The rogue is better killed.

5. Anaesthesia and Euthanasia

For minor operations such as marking the ears or bleeding by cutting the
ether may be used. The easiest way to administer this is to put the mouse
jam jar, covered with a Petri dish or other convenient object and containing
tle ether on some cotton-wool. [Care must be taken to see that the mice
ot get wet with the ether.—*Ed.*]

For major operations one may give intra-peritoneal injections of Tribrome-
ol (Avertin), the dose being 0·1 ml. of a 2·5-per-cent. solution for each
. of body-weight. Alternatively, Nembutal (Abbot) may be given, the
isite dosage being given by the maker.

[Peacock, F. W. (1944) in a private communication from the Glasgow Royal Cancer Hospital, recommends coramine as an antidote in mouse anæs thesia. In cases of asphyxia he has found that spontaneous breathing may b restored in a fair number of mice by the following method : The mouse is held by the tail and swung gently in a horizontal circle with its head hanging down The procedure floods the brain with blood.—*Ed.*]

For euthanasia one may use chloroform, but if bacteriological work is t be done it is better to use coal-gas. Both are quick and painless. Som people are very skilful at breaking the neck. When this is well done, deat is instantaneous, but the trachea and other organs may be ruptured an pathological examinations thus spoilt.

[Berenblum, I. (1944), in a private communication from the Sir Willia Dunn School of Pathology, University of Oxford, recommends that the mou be held by its tail, in one hand, and by its head immediately behind th condyles (occipital region) in the other. With a sudden jerk apart the cervic cord is severed. Some involuntary movements may occur for a few secon but the method is claimed to be completely reliable and very human Kennaway, E. L. (1945), in a private communication from the Chester Beat Research Institute, suggests the following method : " If one takes a mouse b the tail and puts it on a surface that gives it a good foot-hold, it nearly alwa places itself in the best possible position for killing, which is done by laying penholder or pencil across its neck and dislocating by a sharp pull on the ta If, as sometimes happens, the mouse struggles violently and does not put its in the proper position when held by the tail, lift it by the tail, strike its he *hard* on the edge of the table ; this stuns it and dislocation of the neck described above is then quite easy."—*Ed.*]

6. Some Observations on the Diseases of Mice

By P. A. Gorer

General observations. We have no reason to suppose that the diseases mice are any less varied in their manifestations than are those of man, and the is a vast literature on the subject. The most up-to-date account is to be fou in the *Biology of the Laboratory Mouse*, written by the staff of the Roscoe Jackson Memorial Laboratory and edited by Snell (1941), which is strong recommended to all who require detailed information. Here we will lim ourselves to an attempt to outline the signs and symptoms of those conditio with which we have met and of conditions of great epidemiological importanc

Attention has already been drawn to the vital importance of gene prophylaxis. So far as possible the breeding-room or rooms should be clos populations, wild rodents being excluded and mice from other laborator being introduced only with very great caution. If animals are obtained fr an Institute in which one can have great confidence one need not fear a major epidemic, nevertheless the strangers may carry organisms to which t original strains are not immune and a mild outbreak may occur. Grüneb (1943) suggests that newcomers should be isolated and perhaps crossed w one or more resident pure lines or other type of resident strain. These v serve as good indicators of any infections that may be carried. Very gr circumspection should always be exercised with mice from commerc breeders.

The detection of carriers may be very difficult ; cultures of the urine, fæces d various viscera, especially the spleen, may be needed. Enlargement of spleen with or without enlargement of the lymph nodes is a very suspicious n. Normally the spleen weighs from 50 to 100 mg. and is not palpable ough the abdominal wall. Even a moderately enlarged spleen may be pated under light ether anæsthesia. The easiest lymph nodes to palpate just above the folds of the groin. The axillary nodes may also be palpated, mice do not like being handled in this region. Generalized enlargement of lymph nodes may be leukæmic or due to a chronic infection. I have seen n chronic infection with *S. typhi murium*, an organism to be avoided at all ts. A differential blood count may be helpful ; normally there are from to 90 per cent. of mononuclears in the blood and a polymorph count of more n 40 per cent. should make one suspicious of some infection. We should phasize that all these signs may be found in mice that are apparently in fect condition in other respects.

However many precautions are taken, there are bound to be minor out-aks of disease. In order to assess the general physical condition of mice it ssential to pay attention to age. The potential longevity differs greatly ongst different pure lines ; in some, most mice die by about 18 months of but in general they may live about 30 months, exceptional mice living ears or more. Old mice may show de-pigmentation of the coat or con-rable loss of hair. They show a variable loss of vigour and may become y emaciated. Death may be due to tumours, of which a large variety have n described, and renal disease is a common cause of death. Whilst cardio-cular diseases undoubtedly occur, they have not as yet been adequately cribed.

The condition of young mice is best assessed by the coat, which should be oth and glossy. A sick mouse usually sits hunched up and has a ruffled ; the eyes may be half shut and weeping or possibly purulent. Another ul indication is to hold the mouse by its tail and place the forefeet on the -gauze lid of a cage. A healthy mouse catches on hard and tries to pull y. A certain amount of experience is needed to recognize the strength of , and the varying temperaments shown by different mice must be taken into unt.

From time to time one or more mice may be found dead in a cage and it to be decided whether this is likely to herald a dangerous outbreak. One , of course, kill all survivors and have the cage autoclaved, but there may many excellent reasons for avoiding such extreme measures. A bacterio-cal examination of the heart-blood, liver and spleen is often desirable. " A simple method for the aseptic dissection of mice," see Himmelweit, F. 4), *J. Path. Bact.*, **56**, 271–272.—*Ed.*] The commonest causes of casual hs I have found to be coliform bacilli, Morgan's No. 1 bacillus, *Proteus* and ram-negative bacillus that is probably a member of the Pasteurella group. ntion must, of course, be paid to the morbid anatomy and local findings, significance of some of which will be given later. In many cases the r may be conspicuous by their absence and there may be some risk in ing for the results of bacteriological examination. One should pay careful ntion to the condition of the other occupants of the cage ; if they show icious signs it is best to eliminate them. If their condition appears good ay be safe to leave them and inspect them twice a day. Very often no

further deaths occur. In cases of doubt, isolation should be enforced a once.

Arthropod parasites. Detailed notes on the control of these parasites wi be found in Chapter 3. With special reference to the mouse it should be noted (*a*) That more than one species of flea is capable of infecting mice, and tha their presence, since they breed not on the animal but in the bedding, is a sur indication of inadequate hygiene. (*b*) That bed-bugs are specially liable t occur if wooden cages are used. (*c*) That four species of mite have bee described as infecting the species. (*d*) That the commonest louse is *Polypla serrata*.

Helminth parasites. Probably all mice harbour parasitic worms in the alimentary canal, but these usually produce no symptoms so that most worke are unaware of their presence.

The worm that is most commonly recognized is the cystic stage of *Tæn tæniæformis*, also known as *T. crassicolis*. The adult worm occurs in cats an may reach mice from the cat's excreta via the sawdust. It is extreme common and usually produces no symptoms. The cysts occur in the live usually singly, but occasionally two may be present. The larval worm ma die and the cyst become fibrosed. Occasionally it may become infected and fatal septicæmia result.

For further details on helminth infection the reader is referred to Heston article in the *Biology of the Laboratory Mouse*.

Protozoal infections. A number of saprophytic protozoa may be found the mouse and probably some of these can cause disease under certa circumstances.

Klosiella muris is a sporozoon affecting the kidneys. It is extreme common in older mice in certain colonies. In affected individuals the kidne are slightly enlarged and have a characteristic mottled appearance. T parasite undergoes schizogony in the renal cells and the spores escape in t urine. Other organs also may be infected. Once the parasite has real become established there is no known means of eradicating it. It must distinguished from various non-infective renal lesions. In these the kidne are usually shrunken and scarred or possibly cystic, the characteristic mottli is absent, and the kidneys are either brownish or normal in colour Histolo ical differentiation is easy, the parasite being easily visible on section. If has been detected all mice in the cage should be killed and the cage should autoclaved. If this is done promptly there is every chance of checking t spread of the disease.

Sarcosporidiosis is easily recognized at autopsy by the presence of fine whi streaks in the muscles. As a rule there are no symptoms before death, althou sometimes the mice appear unusually sensitive on handling. It is most erra in its appearance. I saw it about ten years ago and it disappeared without a special measures being taken ; during the last few months one or two cas have been seen again. Other workers have had similar experiences.

Diseases due to bacteria or viruses. In this section no attempt is made completeness ; precise diagnosis usually depends upon adequate bacteriologi investigation and a consideration of the pertinent methods does not fall with the scope of this article. The most that can be attempted is to describe certa important epidemic diseases and to give guidance in the interpretation of vario symptoms and pathological findings.

Before proceeding further it is desirable once again to stress the importance prophylaxis. The damage done by a severe epidemic cannot be assessed tirely in terms of immediate mortality. Carriers are likely to be left behind, id in unfavourable circumstances the epidemic may break out afresh. A rther peril has been elucidated by Topley and his co-workers. An epidemic ay die out in a given population which may then appear to be completely mune to the infective agent. If, however, healthy un-immunized mice are troduced a new outbreak is likely to occur, affecting first of all the new-mers but later on the original stock as well. This phenomenon is of con-lerable practical importance. Another common phenomenon is that a latent fection, e.g. with various viruses may be lighted up in an apparently healthy ock by experimentation.

ouse typhoid and other septicæmic diseases.

Mouse typhoid is probably the most important epidemic disease affecting e mouse. It is caused by members of the Salmonella group, usually *S. typhi urium*, but sometimes *S. enteritidis*. These bacteria not only will cause a rrible mortality amongst mice, but may give rise to severe illness in man. ce and other wild rodents are undoubtedly carriers and it is also possible at flies may act as transmitters.

The natural disease in mice may run a course of variable acuteness. In e acute disease there are no specific symptoms, the affected individuals nply showing a rough coat, conjunctivitis, etc., that may result from almost y infection. There may be diarrhœa, but the stools are usually well formed ough somewhat paler than usual.

Death may occur in about seven days in the really acute cases. The rbid anatomy under such circumstances is of little help in diagnosis. The estines may be engorged and possibly ulcerated. If the animal lives long ough the spleen becomes enlarged and the pulp shows the presence of merous myeloid cells. Similarly there is a variable degree of hepatic largement, with or without the presence of miliary abscesses.

The more chronic cases show a variety of physical signs. Some may develop lastic peritonitis. The liver and spleen may show a " sugar-icing " deposit their surface, whilst the intestinal coils are matted together, though the last ding is rather unusual and there is more often considerable ascites. On ner occasions there is a generalized glandular enlargement that may resemble kæmia. Indeed the present author has been sent supposedly leukæmic ce that were in reality suffering from chronic Salmonella infections. A very mmon finding is splenic enlargement without any other obvious sign.

Diagnosis depends upon bacteriological findings. Prophylactic measures ould be ruthless once a diagnosis is made. Survivors of an epidemic prove be carriers in the vast majority of cases.

Pasteurella muriseptica may also give rise to a septicæmic disease assuming demic proportions. Experimental infection with the organism gives rise to urulent pericarditis which is an uncommon finding in mice. Unless cultures made shortly after death the infecting organism may be overgrown by cal bacteria, especially *B. proteus*. Various other infections have been scribed ; Morgan's No. 1 bacillus has been known to give rise to serious demics, but in the experience of the present author it is not of grave nificance.

Those who have not adequate bacteriological facilities should bear in mind the advice given on p. 161 concerning the assessment of mortality amongst stock mice.

Infectious ectromelia, although first described by Marchal as recently as 1930, is a fairly common epidemic disease ; it is caused by a virus. In very acute cases there are no obvious anatomical features. If the disease is less severe, the first sign is usually enlargement of a foot, usually a hind foot; vesicles may form, and crusting over superficial ulcers is likely to occur. Gangrene may supervene. The condition may remain limited and recovery occur, or the gangrene may spread with fatal results. Diagnosis may be established histologically by the presence of inclusion bodies in the epidermis, intestine or pancreas. Survivors may prove to be carriers and recurrent outbreaks may occur. Mortality may be very high. The source of infection is usually unknown, but wild mice are obviously suspect. [Focal areas of necrosis in the liver are characteristic lesions.—*Ed.*]

Senile gangrene may resemble ectromelia. However, the age of the affected animals and the absence of a preliminary œdema should make differentiation easy. In addition senile gangrene is usually confined to the tail and toes and the animal in other respects usually appears healthy.

Polyarthritis due to *Streptobacillus moniliformis* may also cause confusion with the above condition. Involvement of the heart and pericardium is common as well as the arthritis. The organism may be cultured on media enriched with serum.

Respiratory diseases. Various epidemic pneumonias and catarrhs have been described. They are usually typified by wheezing, "chattering" and other signs of respiratory distress such as abdominal breathing. Any of these signs are liable to be seen in a large colony of mice and it is important to know how to assess them. The characteristic wheezing chatter is easy enough to hear but may be very difficult to locate. Sometimes a mouse may make a surprising noise when asleep which will apparently stop as soon as it is disturbed ; however, if such mice are held close to the ear the abnormal breathing may still be heard.

If the other mice in the cage and the affected mouse appear quite healthy no anxiety need be felt. If the latter is in obvious respiratory distress it should be killed and autopsied. One may find that some neoplastic condition is responsible, or there may be pneumonic consolidation. Acute respiratory distress may be caused by intestinal dilatation, a condition that is often fatal but rarely, if ever, gives rise to even minor epidemics. Very often one may be astonished to find no obvious anatomical abnormality. If the heart and roots of the lungs are sectioned, one may find the larger bronchi partially obstructed with laminated or hyaline bodies which stain pink with eosine. Their nature is unknown at present ; they may be derived from the bedding.

In general, respiratory signs need not necessarily be regarded as grave, if present alone, but inexperienced workers would probably do better to err on the side of caution when in doubt.

[*Editorial Note.*—Reference should be made to the important pneumotropic virus infections of mice. As R. E. Glover (1945) points out in a private communication it is probable, although not proved, that all these infections were latent in mice and were activated by the intranasal inoculation of material of human origin. Thomas, *et al.* (1944), in their paper on the activation of **mouse pneumonia virus,** *Proc. Soc. exp. Biol.*

Y., **55**, 1, were able to incriminate human serum. Other important references are those Francis, T., Jr., & McGill, T. P. (1938), on **meningopneumonitis virus,** *J. exp. Med.*, , 147–160; of Sulivan, *et al.* (1939) on **pleuropneumonia,** *Proc. Soc. exp. Biol., N.Y.*, , 620; of Horsfall, F. L., Jr., & Hahn, R. G. (1940), on **pneumonitis,** *J. exp. Med.*, , 391; of Hemberg, *et al.* (1940), on **latent pleuropneumonia,** *Z. Bakt.*, **146**, 129; of einzmann, K. (1941), on pulmonary disease in white mice, *Klin. Wschr.*, **20**, 910; of unnert, R. (1941). on **bronchopneumonia,** *Z. Bakt.*, **147**, 161; of Karr, Helen V. 943), on **latent pneumotropic virus,** *J. infect. Dis.*, **72**, 108; and of Nigg, C., & Eaton, D. (1944), on the isolation from normal mice of a **pneumotropic virus,** forming elentary bodies, *J. exp. Med.*, **79**, 497–510. Andrewes, C. H., & Glover, R. E. (1946, *it. J. exp. Path.*, **26**, 379) have recently described a virus disease (**"grey lung" virus)** ich appears to be distinct from the mouse-pneumonia viruses hitherto described. r a paper on "Infection and immunity in **mouse catarrh,"** see Pearson, H. E. (1942), *Bact.*, **43**, 229–235. For the relationship between infectious ectromelia and vaccinia Burnet, F. M. (1945), *Nature, Lond.* **155**, 543].

Hepatitis. Diseases of the liver are by no means rare in mice and may company a number of infections. Sporadic cases usually show abdominal largement due to ascites. The liver may be variably enlarged and granular d may possibly show yellowish flecks. There may be a whitish "icing-gar" deposit on liver and spleen. The course of the disease may be very g with the animal in a fairly good general condition. If this occurs in a ent arrival in the colony it should be treated with the greatest suspicion. the worker is satisfied that his general prophylaxis is good and he knows his ains well, a more conservative view may be taken. All the same, the affected use is better killed and its neighbours should be watched.

An interesting type of hepatitis is caused by *Bacillus piliformis.* The ease was first described by Tyzzer in 1917, who found it was fatal to the panese waltzing mouse (*Mus bactrianus*) but apparently not to ordinary oratory mice. Recently Tyzzer has shown that the organism may be carried strains of the common mouse (*Mus musculus*), a fact confirmed by the sent author.

In my own experience the organism is not likely to give rise to widespread demics if simple prophylactic measures are taken as soon as a case is seen. chief drawback is that it may be quite unsuspected until one starts some eriment, in which case clinical manifestations may occur. Thus in one eriment hepatitis occurred in all mice that had developed skin tumours er treatment with methyl cholanthrene. Vigorous prophylactic measures m to have eradicated the organism but one can never be quite sure of this.

Infected mice are usually in obviously bad condition and have diarrhœa. autopsy the liver is the only organ with visible lesions. It may or may not enlarged but there are always yellow necrotic spots on it. As a rule these roughly circular in shape, but in severe cases they may be confluent. zzer showed that the organism is present in the intestine and the present hor has shown that lymph nodes are infective.

The organism has not been maintained in culture and must be recognized ologically. It is a long filamentous bacillus that can be seen in groups hin the liver cells in ordinary sections stained with hæmotoxylin and eosine. rapid diagnosis is desired, smears of the liver may be made and stained with m's stain (the organism is Gram negative) or with a Romanowsky stain.

Prophylaxis must be vigorous; all contacts should be killed or well isolated, the cage should be autoclaved or thoroughly sterilized by some other ctive method.

Neoplastic conditions may be very difficult to distinguish from hepatitis and the differential diagnosis cannot be discussed here. Enlargement of the liver in young mice should be assumed to be infective unless the observer is sufficiently experienced to establish the diagnosis.

Diarrhœa may be due to dietary deficiency, but should always be treated as potentially dangerous. If it is confined to mice about 4 weeks old the diet may be suspected, but otherwise the possible presence of some infective cause should be investigated.

Signs indicative of disease of the nervous system. Certain viruses are known that are capable of affecting the central nervous system of mice, but although they may be of importance to the experimentalist they have not been known to give rise to widespread fatal epidemics. Paralyses may complicate various infections, notably that due to *Streptobacillus moniliformis*. Similarly they may be observed in leukæmia and in certain neoplasms.

Rolling is a self-descriptive term and is usually due to infection with the pleuropneumonia organism. In the experience of the present author it is sporadic in occurrence and shows little tendency to spread from cage to cage.

Reference should be made to the fact that mice may be infected with the virus of *lymphocytic choriomeningitis* which is infective for man.

Mange is usually characterized by loss of hair without any general symptoms or loss of fertility. Some cases are apparently due to ringworm fungi of various kinds but the cause in many cases has not been investigated. As a rule the disease is not of serious importance although it is difficult to eradicate. Parish & Craddock described one outbreak due to *Tinea gypseum asteroides* that was transmissible to man. This is highly exceptional and usually the condition shows but little tendency to spread from cage to cage. Loss of hair may be due to lice, whilst skin infections may be due to streptococci and other bacteria. Ordinary methods of prophylaxis must be used.

[Caussé, R. (1942), *C. R. Soc. Biol., Paris*, **137**, 726–727, states that *spontaneous otitis* is encountered in about 1 per cent. of white mice. In contradistinction to its occurrence in albino rabbits, guinea-pigs, rats and certain birds, labyrinthitis rarely appears as a complication.—*Ed.*]

7. A Note on the Training of Assistants

It should now be apparent that adequate supervision of a large mouse colony demands intelligent and experienced supervision. If it is to be done well, it is essential that it should be a full-time task for a senior technician of the highest grade. Too often the care of animals is left as an overtime job for the unskilled or half-trained laboratory assistant. Animal attendants hold a key position in any biological department and should have the remuneration and status that this demands.

It takes from two to three years to train a good assistant, and it is usual to start with young people of fifteen or sixteen. They will never become good unless they are genuinely sympathetic with and interested in the animals, and in addition they need to be observant, intelligent and conscientious.

A great deal must depend upon the attitude of senior members of the staff. It is essential that mistakes should be admitted without delay, and this should be borne in mind when disciplinary measures are being considered.

The best assistants sooner or later take an intelligent interest in the experi

nts, if they are allowed and encouraged to do so. In accustoming them to l with mice, it is quite a good plan to put aside a cage of unwanted animals be handled from time to time. Holding for injections, etc., usually causes ne difficulty. Confidence may be gained more easily if the mice to be held lightly anæsthetized on a few occasions. An assistant who remains afraid he animals will never be of any use.

[*Editorial Note.*—An adequate bibliography of the mouse as a laboratory animal ld fill this volume. A few references have been quoted in the text on pp. 155 and -165. The text-book edited by SNELL, G. D. (1941), *Biology of the Laboratory Mouse* he staff of the Roscoe B. Jackson Memorial Laboratory (Philadelphia : The Blakiston and that written by GRÜNEBERG, H. (1943), *The Genetics of the Mouse* (Cambridge versity Press), provide keys to much of the relevant literature. Current references be obtained from (American) *Chemical Abstracts, Animal Breeding Abstracts, British mical and Physiological Abstracts, Bulletin of Hygiene, Nutrition Abstracts and Reviews, erinary Bulletin* and other abstracting journals. Many titles of importance are listed *ndex Medicus* and *Index Veterinarius*.]

CHAPTER 9

THE WILD HOUSE-MOUSE

(*Mus musculus*)

By Ursula Philip, Ph.D.
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The wild *Mus musculus* must have been reared in captivity by other peop but I have been unable to find an account of the methods employed with t library facilities available at the time of writing. There has been little occasi to use animals from the free-living population on any large scale for laborato work since there are available many tame strains which are identical with the from a systematist's point of view.

The wild *Mus musculus* differs from the laboratory *Mus musculus* in behaviour. The wild animals are very shy and cannot be handled witho some difficulty. When kept in a cage they try to escape at any opportuni and when held by their tail they bite ferociously. This behaviour patte seems on the whole to be dominant. The first-generation hybrids betwe wild and tame mice, irrespective of the way the cross has been made, are almo as shy as their wild parent. In subsequent generations they become easier handle. This may mean that wildness is not determined by one domina factor, but by several. There is also an environmental component in this ty of behaviour, for it is quite noticeable that the tame parent also becomes shy in the presence of its wild mate. There are, however, no properly-controll data available.

For work in which the mice have to be kept in pairs and the young have be recorded at regular intervals, a method had to be devised which allow handling but involved little interference with the normal life of the anima We therefore designed a cage which was adapted to their special requiremen and which may prove useful in keeping other small mammals which are difficu to breed.

The food consumed by wild mice is the same as that of tame ones. I ha used throughout the diet described by Grüneberg (1943) consisting of a mixtu of oats, wheat and rat biscuits, supplemented by water from a drinking-bott The biscuits were supplied by the North Eastern Agricultural Co-operati Society, Ltd., Aberdeen, and were of the following composition :

	Per cent.		Per cen
Fine thirds (wheatings) ..	19·2	Dried milk (skimmed) ..	7·
Ground wheat	19·2	Salt	0·
Sussex ground oats	19·2	Dried yeast	1·
Ground barley	9·5	White fish meal	4·
Ground maize	9·5	Cod-liver oil	0·
Meat and bone meal (50 per cent. protein)	9·5		

Pregnant females were given an additional carrot. Drinking-bottles we fastened to the outside of the cage. We used a flat 200-ml. medicine bott

ided with a bent glass tube from which the mice could drink. These king-bottles are very cheap and are quickly cleaned but they are liable to lop airlocks. This has to be avoided at all costs, because water is absolutely ıtial for animals kept on a dry diet. There is another type of drinking-e which is made up of a glass bulb with a straight tube attached ; such es are difficult to clean though no airlock can develop. [See Figs. 14 15.—*Ed.*]

he cage is made of stout wire $\frac{1}{4}$ in. apart and measures 8 in. × 8 in. × . It is divided into two compartments by a partition of the same material. pening measuring 2 in. × $2\frac{1}{2}$ in., closed by a trap-door, leads from one partment into the other. The trap-door is operated by a wire loop pro-ng over the top of the cage. Each compartment has a separate lid and vanized-iron floor which is made to slide out.

Vire cages of this type are easy to keep clean but they do not provide their bitants with a retreat. As mice live in holes in the walls of houses and ys retreat into dark corners when alarmed, the cages were supplied with a ng place in the form of an upturned flower-pot with a corner chipped out ovide an entrance. Hay was given as nesting material. The mice spent of the time during the day in this house.

he routine of keeping the animals was as follows : One pair and its aned litter were kept in one double cage. The mice occupied one com-ıent during one week and the other compartment during the next one. nhabited compartment, the floor of which was covered with sawdust, was shed with a tongue-jar filled with food sufficient to last a week and a r-pot with nesting material. After a week the other compartment was red in the same manner. Then the lid of the inhabited portion was d and the flower-pot was removed. For a few moments the mice were too ised to move. This gave just enough time to close the lid before they to jump about in alarm. The trap-door between the two compartments hen opened from the outside and the mice quickly found their way into ew home. Young litters were either lifted into the new compartment or or their parents to collect. The galvanized-iron floor was removed and d. If the mice had to be examined the empty compartment was furnished n empty tin. The mice entering the compartment immediately retreated he tin, which could be closed with a tight-fitting lid, and the animals were etherized and examined at leisure. The tin also proved useful in recap-mice which had escaped.

nder the conditions described both males and females bred freely. A pair ft together whenever possible. The presence of a male did not interfere he successful rearing of a litter.

oughout I did not encounter any special diseases, although there was opportunity for infection since my mice were mated to the tame ones of ouse colony. I am not aware of any diseases found in free-living *Mus lus.* [Lymphocytic choriomeningitis has been recorded.—Ed.]

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CHAPTER 10

THE WOOD-MOUSE

Notes on Breeding *Apodemus sylvaticus*

By the late R. M. Ranson

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The smallest cages that we have used for the successful breeding of *Apode* are 8 in. × 12 in. × 5 in. This size will comfortably accommodate one p The mice may be mated up in pairs or, if larger cages are used, one male m be mated with any number up to six females. The most convenient meth is to mate them in pairs; then the male can be left with the female dur pregnancy and when the young are born she may become pregnant ag immediately. The young can be weaned when they are 14 days old. So as is known the gestation period is 21 days. The young are still very sm when they leave the nest, much smaller than young *Microtus agrestis*, and t are able to get through $\frac{1}{4}$-in.-mesh wire netting; it is therefore advisable keep them in perforated zinc cages or metal cages with bars $\frac{3}{16}$ in. ap Presumably the glass "Topley" cages, rat-size, would be suitable also. twist of hay is placed in the cage the mice will eat part of it and turn the into a nest. A little fresh hay should be added about once a fortnight.

The staple diet is oats mixed with a small amount (5 ml. per kg.) of vitam ized oil comprising 3·5 ml. Avoleum B.D.H. and 5·0 ml. Radiostol B.D made up to 175 ml. with olive oil. [A female *Apodemus* was kept in excel health for seven months on the scorbutogenic diet described in Chapter p. 194. No vitamins were added.—*Ed.*]

Water is fed from specimen tubes with a glass tube having an inte diameter of 5 mm. through the cork; the mice lick the water from the en the tube if it is pushed through the top of the cage. The cork must be tected from being nibbled through the bars by having a zinc shield. The t should be slightly inclined as this enables bubbles to move freely up the t without getting stuck.

Apodemus is extremely difficult to handle. Even when it has been kept a considerable time and is fairly used to being handled it is still rather v and without any warning will give a tremendous leap out of one's hand or of the cage if the lid is removed carelessly.

We have not sufficient evidence about the temperature or the intensit duration of light essential for the well-being of *Apodemus*, but we have had majority of our breeding results in a 15-hour light supplied by a 100-' clear-glass bulb at a distance of 6 feet and in a temperature fluctuating betv 6° and 7° C.

CHAPTER 11

DEER MICE

Methods for the Rearing of *Peromyscus*

By Lee R. Dice, A.B., M.S., Ph.D.

boratory of Vertebrate Biology, University of Michigan, Ann Arbor, Mich., U.S.A.)

. Introduction. 2. Accommodation. 3. Nutrition. 4. Bedding. 5. Cleaning. 6. Handling. 7. Breeding. 8. Parasites and diseases.

1. Introduction

ne genus *Peromyscus* includes a number of species and many geographical of mouse-like rodents. All the forms of the genus live natively on the nent of North America, where they occupy many diverse types of habitat. rtheless, most of the species and races may be reared more or less success- with the same cultural methods.

particular advantage of *Peromyscus* over the house-mouse or house-rat aboratory animal is the comparatively slight animal odour given off. A y of several thousand individuals, if kept in clean condition, does not have ously objectionable odour.

2. Accommodation

ost species of *Peromyscus* are somewhat larger in size than the ordinary -mouse and the animals do not endure crowding as well as does that on laboratory form. Nor do they live or breed well in dark, damp, or ventilated quarters. The cages especially must be well ventilated, and ondition is obtained when they are made mostly of wire screen. However, must be protected against draughts.

e cage that I have found most successful for *Peromyscus* is constructed of creen on the front and sides, while the back and top are of wood. This s placed in an enamelled baking pan, which forms the bottom (Fig. 39). e dimensions of the cage must be adjusted to the size of the available pan. tunately the manufacturers frequently change the dimensions produced is therefore important to have the pan on hand before constructing the f the cage. In Fig. 39 are given the dimensions of the parts needed to uct a cage to fit inside a 12-in. × 18-in. baking pan. The inside dimen- of such a cage will be approximately 10 in. × 12 in. The pan should mless and of good quality material. The bottom should be flat and any or grooves in this part are objectionable.

e wooden pieces used in constructing the cage can be made from any worked lumber. Materials that split when nailed are to be avoided. ooden piece that forms the back of the cage must have its outside lower s rounded off so that they will fit the rounded corners of the pan. The n blocks can be given several coats of linseed oil if desired, but paint ning lead should not be used.

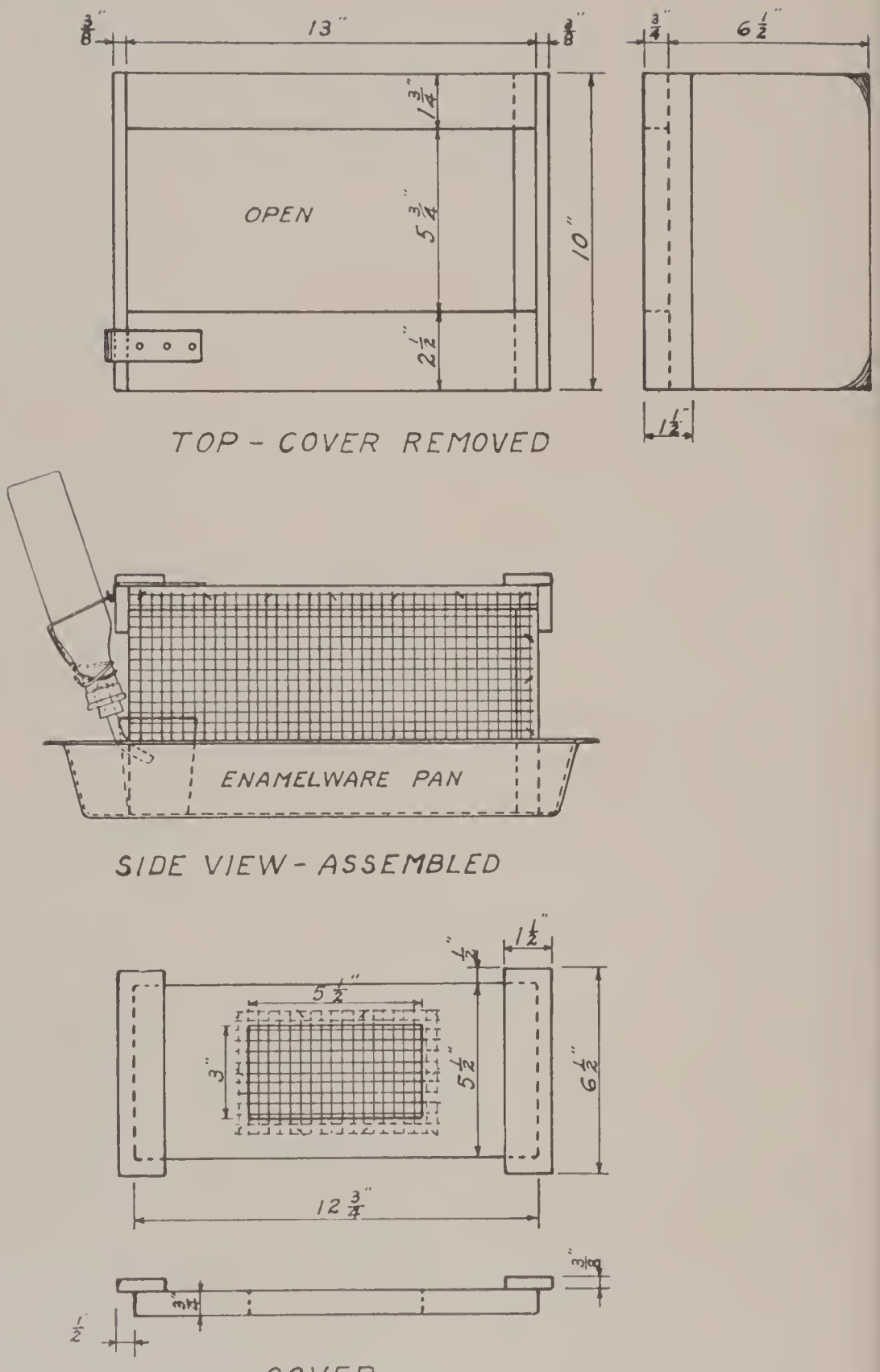

FIG. 39.—DETAILS OF CAGE USED FOR *Peromyscus*.

ıe wire screen is of $\frac{1}{3}$-in. mesh and should be of moderately heavy wire has been galvanized after weaving. Light-weight wire quickly rusts vhile the heaviest grade is too stiff to work easily. To prevent the mice ng the wooden blocks of which the cage is in part constructed, these must ed with metal or wire screen. Strips of galvanized light sheet metal about wide are nailed along each inside edge of the removable top cover. A strip of wire screen is tacked to the lower edge of the wooden piece that the back of the cage. Screen strips are also nailed on the under sides two pieces that, together with the removable top, form the top of the These last-mentioned strips of wire screen should be wide enough to t sideways beyond the wooden blocks, to which they are nailed, for a ce of from $\frac{1}{2}$ in. to $\frac{3}{4}$ in. The strips of wire screen then protect the crack en the wooden strips and the removable cover, when this is in place, and y discourage the gnawing activities of the mice.

assembling the cage it is desirable to allow a clearance of at least $\frac{1}{4}$ in. on de and one end of the top cover, to allow for possible swelling of oards when in use. Before the wire screen is tacked firmly into place hould be taken that the cage stands true and is not warped in any

will be noted that the cage consists essentially of three main parts: ttom pan, the main body, and the removable cover. These parts are gether only by their weight and they can easily be separated for thorough ıg.

supply drinking-water a 12-oz. prescription bottle is hung on the front cage in an inverted position, with a glass nozzle extending through the creen into the cage. The bottle is wrapped in a wire harness, which over a flat metal support fastened to the top of the cage. The bottle ıerefore, be readily removed for filling. The piece of glass tubing that as a nozzle should have an inside diameter of approximately 5 mm. ent slightly in its middle so that one end will project through the wire into the cage. This end of the tube is drawn down in a flame to an g of approximately $2\frac{1}{2}$ mm. The other end is then inserted into the stopper which closes the opening of the bottle. The mice learn imme- to use this sanitary drinking-fountain.

od in dry form is supplied in a glass tumbler placed in the front of the A 4-oz. straight-sided glass is satisfactory, but a larger size may be used ed. This glass is so placed that half of it is inside the cage and the other ıtside. The wire screen of the cage divides the glass into two parts. laced in the outside of the glass passes readily through the screen and ı by the mouse on the inside of the cage. There is, therefore, no need ı the cage for the insertion of food. To allow the glass to be placed in ı a small portion of the wire screen of the front of the cage is cut away, ıg from the bottom, to an amount equal to the space occupied by the ıd bottom of the glass. A tongue of wire screen is left that will project nto the glass. The glass can then be inserted by pushing it upward. the glass not be held firmly in position by the spring of the wire screen, e fastened by an encircling light wire placed near the upper edge of the ıd anchored at both ends to the wire screen.

ectangular opening about 6 in. × 4 in. is cut in the top cover, and this red by a piece of wire screen of $\frac{1}{3}$-in. mesh tacked over the lower side

of the opening. Green vegetables or other kinds of solid food placed on screen can be eaten by the mice as they cling to the underside.

In designing these cages every effort has been made to avoid the neces for opening any cage for the daily care of the mice. Both food and water replenished from the outside of the cage. This arrangement not only gre expedites the care of the animals and thereby reduces expense but it avoids undue disturbance of the animals in the cages. Another import result of not opening the cages is that fewer animals escape. Whenever a is opened certain wild individuals are likely to make a dash for freedom, ei escaping or perhaps being injured in the attempt. Also in the haste of ca for a large colony the lids of some of the cages are likely to be carelessly repla allowing the animals a chance to escape later.

On the front of the cage is attached a brass label-holder that holds a $2\frac{1}{2}$ in. $\times$ 1 in. in size. On this card the numbers of the animals in the cage written, together with any other desired information.

Each cage must be supplied with a nest-box, for these mice are noctu in habit and need a place to retire to during the day. The most conven nest-box is a 1-quart pan, with a hole cut in one side, placed upside-dow one corner of the cage. I have mostly used a 1-quart aluminium dipper the handle cut out. This "igloo" is much appreciated by the mice. I of course, very easily cleaned. The mice attack the aluminium with teeth and ultimately destroy many of the pans. The pans are so cheap, ever, that they are easily replaced.

Racks to hold the cages are essential if many are to be kept in a small sp A wooden rack of which the individual shelves are made of 1-in. $\times$ 2-in. s placed on edge is very satisfactory. The strips run lengthwise of the rack are placed 6 in. apart on centres, open spaces being left between them so any litter thrown out from the cages falls to the floor. If the shelves are 64 in. long, inside of the 2-in. $\times$ 4-in. upright supports, each will hold 5 c The shelves may be spaced 12 in. apart up and down on centres. If a spa 5 in. is left below the bottom shelf, it will be possible to care for the cage 6 shelves. This allows space for 30 cages in a single rack. However, the will be somewhat more stable if they are made double, that is, constructe that they are the equivalent of two single racks placed back to back.

3. Nutrition

The food ration on which many thousands of *Peromyscus* have been re at the Laboratory of Vertebrate Biology is composed of the following i dients by weight:

	Per cent.		Per
Rolled oats	52	Hemp seed	
Dry meat scraps	20	Millet	
Dry powdered milk	10	Sunflower seed	
Whole wheat kernels	5	Dry powdered liver	
Wheat germ	5	Iodine salt	
Cod-liver oil	2	Powdered calcium carbonate	
Canary seed	1		

ll these ingredients except the sunflower seeds are mixed together to form
ration. The sunflower seeds are fed separately, about one tablespoonful
eek to each cage.

his ration has been developed largely by trial and error, but effort has
made to include all the known essentials of diet for a mouse. No claim is
that it is a perfect diet for *Peromyscus*, but at least most species thrive
Some slight changes have been made in the ration from time to time,
or over ten years there has been little modification. During the war it
impossible to secure certain items, especially some of the seeds, and
were omitted without apparent harmful results. For many years I
ttuce leaves (trimmings) twice a week to each cage, but for a number of
now these have been omitted with no apparent decrease in the health or
ty of the mice. The mice seem to thrive in the complete absence of any
lent food, though they eat such food readily when it is supplied.

or mixing the food for our large colony a small-sized cement mixer fitted
an electric motor is employed. The cod-liver oil is first placed with the
oats and these items are well stirred together before the rest of the
ials are added. It will be noted that all the food materials, except the
wer seeds, are of a size that will pass readily through the wire screen in
od dish. The mice particularly enjoy the grains, but they are inclined to
them in their cages. The oats are fed in the rolled condition in order to
the accumulation of oat husks in the food dishes.

ie food dishes on the cages are replenished with the ration every alternate
and a surplus of food is always provided, because these mice quickly die
y become hungry. A metal flour scoop forms a suitable utensil for filling
od dishes. A few mice form the habit of digging the food out of their
. They may then either allow it to become soiled on the bottom of the
or they may store it in one corner. When this habit is noted a card is
l in the dish and no more food supplied to that cage until the material on
has been utilized.

4. Bedding

ie nesting material supplied to the mice is a good grade of cotton batting.
dful of this placed in the cage will quickly be carried by the mice under
st pan and a cosy nest constructed. Care must be taken to ensure that
otton does not come into contact with the nozzle of the water-bottle,
vise all the water will be drained into the cage.

me absorbent material should be placed on the floor of the pan to absorb
and other liquids. The best material for this purpose is peat moss.
ist may be used but it is often very dusty. About a pint or less of the
otive material will be adequate unless the cage is occupied by a large
.

5. Cleaning

e cages should be cleaned at least once per month. During summer a
hat shorter interval is desirable. When a cage is to be cleaned, the mice
e changed to an already clean and dry cage. The dirty cage is then taken
es and scrubbed under water with a coarse brush. Laundry tubs of large
e desirable accessories for use in washing the cages. After the soiled
nd bedding have been removed from the dirty cages it is desirable to

soak the bottom pans, nest pans, and food glasses for a time in water in or to expedite the cleaning process. An ounce of trisodium phosphate twenty gallons of wash water aids in the removal of dirt. About four oun of kerosene floating on the top of the water in the wash-tub aids in the cont of any external parasites that may be hiding in the cages. Our colony *Peromyscus* has remained unusually free from infectious disease and we seld use any other disinfectant.

6. Handling

The mice are always handled with a 12-in. thumb forceps, of which each is covered by a short piece of thin rubber tubing. The mice are picked up the tail, near the base. If they are seized near the tip of the tail the tail-she is likely to be injured or to slip off completely leaving the vertebræ exposed

In handling mice that are somewhat wild, the lid of the cage is slid leng wise to expose an opening of only a few inches. This opening can be protec by the spread fingers of the left hand, while the forceps, held in the right ha are inserted in the cage and the mouse is secured.

7. Breeding

The mice that are to be bred are usually kept in the cages by pairs, each p consisting of a single male and single female. The male need not be remov from the cage when a litter is expected. If a male is present in the cage female will usually become pregnant again during the twenty-four hours lowing parturition. The length of an œstrus cycle is from 4 to 6 days (Cla 1936). The period of gestation of most species of *Peromyscus* averages ab 23 days, but if the pregnant female is nursing young the gestation perio prolonged, sometimes to as much as 30 days or more (Svihla, 1932). young are well cared for by the mother. The adult male if present in the sa cage causes them no trouble. The eyes of the young mice open at ab 15 days of age and at that time they begin to take solid food. The young most species continue, however, to nurse until the age of about 30 days, which time they should be removed from the mother and placed in anot cage. However, if a second litter has arrived before that time, the first lit should immediately be removed to avoid overcrowding; otherwise some the newly arrived young may be killed. The young of the *Peromyscus t* group mature more slowly than those of other species of the genus, and sho not be weaned until they are at least 35 days of age.

The cages that contain mated pairs are inspected at least once a week the presence of litters. The inspection can rapidly be carried out by lift up the nest pan and exposing the nest. A person skilled in estimating the a of the young mice can calculate the date of their birth to within a day. T date of birth is then marked on the cage label.

In order to know which cages to examine during the weekly inspecti coloured strings are hung on the handles of the cage pans. A cage of unma mice has a white string, a mated pair has no string at all, and a pair wit litter is indicated by a red string.

Young females of most *Peromyscus* species become sexually mature at average age of about 50 days, but the females of the species *polionatus* aver

30 days of age when they become mature. Most of the young *Peromyscus* es become sexually mature before the age of 60 days, and many individuals ain motile spermatozoa at much younger ages (Clark, 1938). It is, there- desirable to separate the sexes at the time when the young are weaned. animals are in their highest reproductive vigour at ages from about months to one year, but much older animals continue to be fertile. One of deer-mice produced a litter of young when both parents were over four s of age. The oldest known age to which a *Peromyscus* has lived is eight s (Dice, 1933).

ighting sometimes occurs among these mice, especially if a strange mouse troduced into a cage. The stranger will always be attacked and often will illed, no matter of which sex it may be. When a male and female are g mated both individuals should be placed in a clean cage which, therefore, range to both. The same precaution should always be taken when iduals of the same sex are put together. To avoid overcrowding no more four individuals should ever be placed in the same cage.

ach mouse may readily be marked with its individual serial number by hing holes at various positions in the ears in accordance with a code. operation is best carried out under ether anæsthesia at the time the mice eaned.

8. Parasites and Diseases

xternal parasites are sometimes introduced into our colony by new animals in the field. Such parasites seldom become a serious problem if the cages kept clean. Sometimes, however, a particular cage becomes seriously ed with fleas, scab-mites or sucking lice. When such a cage is discovered e animals in it are sprayed with kerosene and transferred to a clean cage. old cage is also well sprayed with kerosene and later washed. The spray rosene is drastic treatment and should be applied only lightly to the mice, nough should be used to make the fur appear slightly damp. Mice so ed should be kept in a warm room, out of draughts, until they have become ughly dry. None of the mice so treated has ever shown any evidence of burning or hair-loss due to the spray, but some individuals appear sick few days, evidently because they have inhaled the kerosene spray or have the oil off their fur.

e have had very little trouble in our *Peromyscus* colony with infectious es. On the contrary, all our animals are exceptionally healthy. How- these mice undoubtedly will prove to be susceptible to many of the es that affect other laboratory rodents. One will, therefore, be wise not pose *Peromyscus* to possible infection from other types of laboratory ls.

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CHAPTER 12

THE COTTON RAT

(*Sigmodon*)

Production of Cotton Rats in Captivity

By D. B. Meyer, D.V.M., and Martha Marsh, M.S.

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1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Losses. Appendix: Experience in breeding cotton rats at the National Institute for Medical Research, Hampstead.

1. Introduction

In November, 1939, after the announcement by Armstrong (1939) that t cotton rat could be infected with the poliomyelitis virus, the Bureau of Labo tories, Michigan Department of Health, started a breeding colony with w stock (Anthony, 1928) of *Sigmodon hispidus hispidus* (Fig. 40) caught Florida and South Carolina and *Sigmodon hispidus littoralis* caught in Flor and Georgia.

Fig. 40.—Female Cotton Rat, One Year Old.

The colony was started with approximately 500 wild rats. At pre 275 pairs of breeders are producing an average of 950 young each month.

2. Accommodation

A cotton rat colony can be housed in any quarters suitable for the raisin other small animals. Although they seem to tolerate considerable variat in room-temperature we find the optimum to be 65° to 70° F.

A cage (Fig. 41), which was especially designed and built for cotton rat eeding and which has proved very satisfactory, is 20 in. × 10 in. × 10 in. Is constructed of sheet iron and $\frac{1}{4}$-in. hardware screen coated with aluminium

FIG. 41.—COTTON RAT BREEDING-CAGE.

nt, and has an enclosed nesting compartment with a removable pressed d bottom. The exercising and feeding compartment has screen sides and emovable screen bottom. A tray containing wood-shavings slides under

G. 42.—CAGE USED FOR WEANED COTTON RATS AND FOR THOSE READY FOR TEST.
ctures taken by George W. Jennings, Staff Photographer, Michigan Department of Health.)

compartments. The breeding-cages are grouped in units of 25 on movable racks.

Young animals following weaning are kept in small-animal laboratory cages, . × 12 in. × 10 in., which are stacked in batteries of four and mounted on er-tyred trucks or bases (Fig. 42).

A very essential piece of equipment is a flowing-steam chest in which t sterilize cages and other equipment.

3. Nutrition

Originally the diet consisted of a wide variety of feeds including whole o rolled oats, sunflower seed, tomatoes, apples, carrots, fresh green alfalfa, an dried skim-milk tablets, supplemented with commercially prepared fox an mouse pellets. However, during the past eight months we have been feedin about one-sixth of the breeders on nothing but the two commercial feed supplemented two or three times per week with skim-milk tablets, carrot apples or fresh green alfalfa and as yet we are unable to note any variation i the health or production of this group as compared with those still on the varie diet. The advantage of feeding the prepared pellet alone is that by the use o a wire basket feeder we avoid heavy feed loss, simplify the feeding task an greatly decrease the risk of feed contamination.

Water is provided by means of a hanging-drop dispenser.

4. Breeding

Six-weeks-old cotton rats of healthy and sturdy stock are selected fo breeders, and are permanently marked by a system of ear and toe marking. At the time of pairing a breeding record is started for each male and femal This record includes information regarding sex, generation from wild stoc identification number, date of birth and production record.

Under the present system of management, matings of a male and a fema are permanent except in certain instances when it is found possible to rota one male among several females.

Male and female rats from 6 to 7 weeks of age are sexually mature and a ready to be paired. In studying vaginal smears from a limited number females, we have found that the œstrus cycle is irregular, varying from 4 to days, with an average of 5·7 days. The period of gestation varies from 26 28 days. The average number per litter was 5, with 12 the maximum that ha been encountered. Newly-born animals have averaged 7 g. in weight and a well haired out at birth. They have begun to run around the cage at an ear age, have eaten solid food within about 1 week, and have been ready to b weaned at 3 weeks of age. Meyer & Meyer (1944 *a*) have recently report the following averages: œstrus cycle 7·0 days, gestation period 27 day number of births per litter 5·6, and weight at birth 6·8 g.

The average production span of a female cotton rat is not known. Th oldest rat (No. C16) in the colony that is still producing is 18 months ol This female has a record of 116 births in 16 litters. However, it is not unusu to discard females after one or two parturitions because of subsequent failure become pregnant within 60 days.

Precautions must be taken in the handling of cotton rats and it is advisab not to disturb the newly-born. The tenth-generation rats, born in the colon are as nervous and likely to bite as are rats when first caught. Heavy cowhi gloves must be worn when handling rats two weeks or more of age. The anim should be grasped firmly at the nape of the neck, as handling the rat by its t usually results in stripping off the skin. [Schwentker (1943) has succeeded producing a strain of cotton rats more amenable to confinement.—*Ed.*]

Through a system of close inbreeding it may be possible to produce a specific ain of cotton rat more uniformly susceptible to viruses. From a group of brother-and-sister matings three pairs were selected to be used as the basis hree pure strains of animals. Two of these strains have been discontinued le the third, which showed evidence of lighter hair coloration, has produced th-generation rats of sturdy stock and through selective breeding is showing roduction record significantly higher than the average.

5. Losses

Each month we have experienced 10 to 15 per cent. mortality among the ding and weaned stock and an average loss of 15 per cent. of sucklings, uding those dead at delivery. The majority of the losses in the breeding k were due to fighting, improper handling and starvation. Of the total hs 45 per cent. have been due to fighting. The loss has been roughly er cent. among males and 40 per cent. among females subsequent to their ing or parturition. This loss of breeding stock has been materially reduced vashing each member of the pair with a 1-per-cent. pine oil or coal tar fectant solution in order to destroy their natural odour. A small unknown entage of animals died as a result of injury during handling or have had to killed because of severe injury inflicted by cage mates. Cotton rats rently are not very hardy and easily die as a result of moderate under- ng. If in cages where large numbers of rats are kept the food-supply is itted to become low over week-ends, the rats will become weak and e to eat, and some of them will die.

osses due to bacterial infections and internal parasites have been small. eriological examinations made on rats that died from unknown causes have led the presence of a variety of bacteria including green-producing tococci, staphylococci and organisms of the Proteus group. Cotton rats vidently not very susceptible to organisms of the Salmonella group, since olony at one time was housed with a small colony of mice in which Sal- lla infection was present. Furthermore, cotton rats experimentally have ed artificial oral exposure to Salmonella organisms.

nternal parasites have been recovered from wild animals which died g or soon after shipment. Of 170 wild animals thus examined, 49 were d to be infested with either tapeworms, roundworms, echinococci or filaria. Fleas and lice also were found on the wild stock. No parasites y type have been found in any of the animals that have been raised in the y.

hile our losses of rats of all ages has been significant we have never ienced anything of an epizootic nature, the death-rate being more or less m from month to month. However, a research laboratory to which we hed 50 or more per week was compelled to discontinue its studies because epizootic in the colony. Our quest for the causal agent involved in this vas prematurely halted because of the advisability of complete disposal e colony and thorough cleaning of the colony quarters. However, we able to conclude to our satisfaction that a virus was involved. Briefly story was that of a very acute, highly virulent infection which resulted to 95 per cent. mortality within 3 to 6 days after the rats had been placed colony quarters. After the disposal of all animals and a thorough cleaning

of the room and sterilization of equipment, the project was being resume without further evidence of the infection.

At various times during the history of the colony we have observed alopec (loss of hair) among the rats. Alopecia was first noticed in the dorso-lumb region where the loss of brown-tipped guard hairs resulted in exposure of t dark under-fur. In advanced stages the back became almost denuded of ha (Fig. 43), and the area eventually extended forward to the head region. T condition has been exhibited in other specific regions such as those around t eyes, on the forehead and at the base of the tail. Originally, because of fe

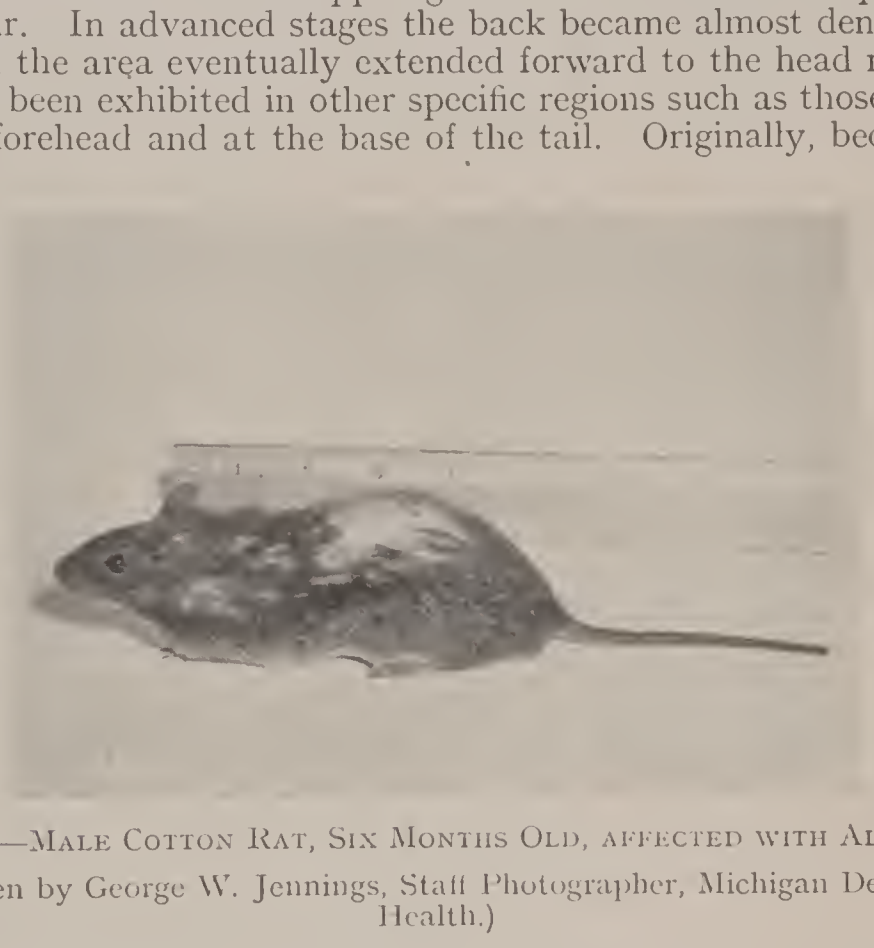

FIG. 43.—MALE COTTON RAT, SIX MONTHS OLD, AFFECTED WITH ALOPECIA.
(Picture taken by George W. Jennings, Staff Photographer, Michigan Department o Health.)

that the condition might have been parasitic or infectious in nature, it was c practice to isolate and treat all affected individuals with various local appli tions. At one time we adopted the policy of destroying all animals wh failed to respond to treatment, but the success of our methods of treatme was questionable since the controls usually showed the same degree of recover as did the treated groups. We now feel certain that the condition is associat with vitamin supply. Cunha *et al.* (1943) reported prompt recovery fr alopecia when inosital was added to a ration of naturally occurring feeds.

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APPENDIX

Experience in Breeding Cotton Rats at the National Institute for Medical Research, Hampstead.

[The following information, kindly supplied by Dr. C. H. Andrewes, F.R.S., cluded since it suggests interesting differences between colonies and may ide valuable information to those working on cotton rats imported from United States. Copies of notes supplied by Mr. Schwentker to the National tute for Medical Research (see list of references) and dealing with the efeller colony, are in U F A W's possession and will be loaned to those ested provided the necessary permission is obtained from Hampstead. .]

ine females and seven males were received from America on 14 July, 1943. n pairs were selected and put into cages on 19 July, 1943.

ages. The cages are galvanized-iron trays 20½ in. × 15½ in. × 6 in. deep a corner bracket on each corner 1 in. from the top inside; on these brackets s a galvanized-iron wire top 20 in. × 15 in. × 7 in. These cages were used ıse they were available, and they have proved very satisfactory. Inside age is placed a 12-in. section of 4-in. drain-pipe (unglazed) as suggested r. Schwentker in his notes sent with the cotton rats.

ne pipe has proved most useful, as the rats use it to rear their young, to in and as a place of refuge when frightened. The pipe is also handy for ferring rats from cage to cage by placing your hands over the open ends. ve space we tried to breed cotton rats in smaller cages, 10 in. wide × n. deep × 6½ in. high galvanized-iron wire standing on a galvanized-iron with a 6-in. section of drain-pipe. At first, instead of a drain-pipe we put

in a ball of wood-wool, but the rats refused to breed until the drain-pipe w put in. The rats are now breeding fairly regularly in the smaller type of ca but the young do not show proper growth and the average litter size is sm compared with that in the larger cage.

Food. At first the cotton rats were fed on a dry diet consisting of wholem flour, dried full cream milk, dried yeast, meat and bone meal, cod-liver with 2-per-cent. mineral mixture. Greenstuff, i.e. lettuce, spinach, cabba sprouted oats was given each day. Cotton rats seem to enjoy greenstu especially spinach, and very rarely is any left in the cage next morning. Lat we have been feeding a cubed diet with a smaller fat content, skimmed m being substituted for full cream. Water is given from an inverted bottle wi a hanging drop tube.

Bedding. For bedding sawdust is used and a piece of wood-wool is put in the cage ; the cotton rats take this into the drain-pipe and make very comfo able nests.

Breeding. At 6 weeks of age those that are selected for breeding are pair off, one male and one female to a cage. By pairing off at this age the situati in which the female attacks the male and kills him is kept down to a minimu As stated before, our batch of rats arrived on 14 July and were paired the 19th. Litters began to arrive on 17 August and since then most of t pairs have produced a regular monthly litter. The young are left with t mother for three weeks when they are weaned. (The first 30 litters averag just under 6 per litter.)

At weaning they are sexed and put into age groups about 12 per grou Fighting takes place among these young cotton rats when put into groups, a deaths occur. We have succeeded in getting over this difficulty to so extent by putting the groups into larger cages, 41 in. × 15 in. × 10 in.

Management. Cotton rats, like ordinary rats, seem very susceptible changes of attendants, and this we have tried to avoid ; also great care shou be taken in choosing the attendant as cotton rats react well to good a sympathetic treatment. A method we use for sexing and sorting cotton ra is as follows :

The drain-pipe is taken from the cage with the rats inside by placing o hand over each end of the pipe. The cotton rats in the pipe are then empti into an ordinary sanitary bin about 24 in. × 18 in. The young and old m then be handled without fear of getting loose, as they just cannot jump two fe We have never used gloves in handling cotton rats this way and very seldo have we been bitten. The time saved in handling cotton rats this way is qui considerable. Lights were turned on in the colony room in the winter by automatic switch for two hours each morning and evening, 6 a.m. to 8 a.m and 6 p.m. to 8 p.m. The lengthening of the day seems to help in keeping the production of litters during the dark winter days.

CHAPTER 13

THE COMMON OR FIELD VOLE

(*Microtus agrestis*)

Based on a publication * and notes of the late R. M. Ranson
(*Bureau of Animal Population, University Museum, Oxford*)

1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Handling and transport. 6. Diseases.

1. Introduction

Voles were maintained as a laboratory stock in the Bureau of Animal ulation at Oxford from 1930 until 1940. The technique described below worked out for keeping them in the best health.

The vole stocks at Oxford played an essential part in the laboratory study natural epidemics, such as those in North Wales and Scotland in 1933 arently caused by *Toxoplasma* infection, which were investigated by Findlay liddleton (1934). Voles have also proved to be highly susceptible in the ratory to a number of virus diseases (see section 6).

The vole has an additional interest, since all existing laboratory stocks e been living under artificial conditions for only a few generations, while usual types of laboratory rodents (white mice, rats, guinea-pigs, etc.) have domesticated for such countless generations that they have developed a ber of characteristics by artificial selection. There is little possibility of selection having had much effect on the vole stocks as yet.

There are supposed to be two races or subspecies of *Microtus* in Great ain—*M. agrestis* (northern) and *M. hirtus* (southern). The existence and ions of the two are still very problematical. The stocks at Oxford were ved from the hills of North Wales and the Scottish lowlands, with some tions from Aberdeenshire and Oxfordshire. They have been referred to onvenience as *M. agrestis*. The question of these supposed races merits er study, since it is now possible to supplement systematic work on tural characters by experiments on physiology and susceptibility to rent diseases.

The activity of voles is not influenced by light or darkness: they have nuous activity and feeding rhythm every 2 to 4 hours throughout the and night. It is, therefore, fairly easy to discover a sick vole by noting mount of grain and water left from the last feed.

The mean expectation of life after weaning of an English vole living under al laboratory conditions at Oxford was about 220 days (Leslie & Ranson, . Only voles aged 3 to 8 weeks were sent out by the Bureau. This was for two reasons: it allowed a maximum period of life in which to plan term experiments; and young voles seem to adapt themselves to change of onment much more readily than older ones.

* Matter taken from Mr. Ranson's paper in the *Journal of Animal Ecology*, vol. 3, 1934, has been incorporated by kind permission of the Editor.

2. Accommodation

Room temperature. If voles are kept in large cages or in communities, an have plenty of hay in excess of their food requirements, so that they can mak nests, the temperature of the laboratory in which they are housed is not ver important so long as it falls within the limits of 0° to 30° C. With plenty cover, voles flourish at temperatures below 0° C. but their water-bottles freez up and break. At temperatures much above 30° C. voles do not thrive in th laboratory, although some will remain alive at 40° C.

If they are kept in small metal cages or glass Topley cages, much greate care must be taken to regulate the temperature of the laboratory so that does not fall below 15° C. For if they are kept in a cold room with only small amount of nesting material to insulate them, they lose heat rapidly become comatose, and die.

Cages. We have found cages 12 in. × 8 in. and 5 in. deep, metal or wooden to be very satisfactory for breeding; while metal cages 10 in. × 3 in. an 3 in. deep with a covered nest-box are suitable for keeping voles singly.

Voles can be kept singly in glass Topley cages, but they are apt to becom rather wild and attempt to escape at every opportunity. The cages a improved in this respect if dark paper is pasted on to the outside of the glass.

Granulated peat-moss is used for litter; it is superior in every way to saw dust for this purpose. If the 12 in. × 8 in. × 5 in. cages are used they w need cleaning out only once a fortnight. If, however, the litter becomes we through the water-bottle accidently siphoning out, the cage should be cleane out at once, for voles do not like sitting on wet litter.

The voles should be kept separately, but if it is found necessary to keep som in communities for a while they should be put into large cages immediately arrival, *with the sexes separate*. If this precaution is not taken and a number adult males and females are put into the same cage together, fighting will resu and some of the animals may be killed.

[The type of cage designed for the Orkney vole by Dr. J. A. Young, s Fig. 44, p. 193, would presumably be suitable for this species also.—*Ed.*]

3. Nutrition

In the wild state voles eat mainly the stems, leaves and roots of rough grass of all kinds (*Holcus, Molinia, Deschampsia*, etc.), and certain herbaceous plant During a vole plague large areas of rough grassland and pasture are complete laid waste, and a dense intricate system of runways, nest-holes and "lav tories" is exposed if the dead grass is removed. In the wild state a vole ea nearly its own weight of fresh green food in a day; to give this large quanti of fresh grass in captivity would be laborious, so mangolds or carrots, hay a mixed corn are given as well.

The following daily ration is given to each vole:

Hay	An excess.
Water	At least 25 c.c. available.
Clipped oats	5 parts } About 8 g., mixed
Wheat or mixed corn *	1 part } thoroughly.

* The mixed corn we have used is 60 per cent. oats, 30 per cent. split and flaked maize a 10 per cent. wheat.

f Topley cages are used there will not be any room for hay, as this will cause vater to siphon out of the water-bottle if it touches the end of the tube. nch of chaff (about 4 g.) given daily may be used instead.

f the larger type of cage is used and there is room for hay, so much the r, for hay is always better food value than chaff, unless the latter is fresh ach day. A large twist (about 50 g.) should be put in and renewed when ssary or when the cage is cleaned out.

he water intake of voles, which in nature are grass feeders, is enormously than that of mice. So that although 25 ml. may appear to be rather sive, it will be found that if voles have to go for more than 24 hours at a without attention (e.g. at week-ends) they will require nearly all this nt, especially if the laboratory in which they are housed has a dry sphere.

Vater is given in mouse drinking-vessels. The most convenient type for sterilization and filling is a small bottle fitted with a rubber bung and a tube. These are supplied, complete, to hold about 65 ml., by R. B. er & Co., at 4*s.* 6*d.* per dozen (pre-war price). The internal diameter of ubes should be from 5·5 to 6 mm. If it is greater than this, a drop will be ed every time a bubble is formed ; if it is smaller the bubble may stick form an air-lock. The bottles are supplied with quite long tubes ; if sary these can be cut down according to the depth of the cage employed. nd of the tube at which the vole drinks should be flamed to smooth it.

4. Breeding

has been shown (Baker & Ranson, 1932, 1933 *a* and *b*) that light and heat ne principal controlling factors of the breeding season of voles, and that perature in excess of 15° C. and 15 hours of natural or artificial light per are necessary if maximum breeding is desired. Factors affecting the ing of the vole in nature have been studied also by Brambell & Hall).

the presence of females, males will not live together but invariably fight death, so that each breeding male has to be kept in a separate small cage nated to two females at a time. Until the temper of each individual male wn, and to guard against death by fighting, it is best to put the male in ating cage after the females have been in residence for about 12 to 24 ; this gives the females time to settle down and claim the cage as their ory, and the excessive pugnacity of the male is thereby balanced. ugh a little fighting is almost unavoidable, the females will not be bullied he case when the male is put in first.

breeding is attempted in large cages, four or six females being put with nale, the same procedure applies except that when females become ant and are removed new females must not be put in to take their place y will very often be bullied and bitten to death by the other members " harem." The difficulty can be got over either by waiting till all the es are pregnant—this wastes space rather when the numbers become low y starting another cage with the remaining few and some fresh females to up the number and treating them as a new unit, adding a fresh male on. This procedure quite overcomes the complex troubles which arise the strong territorial instincts present in voles in captivity.

The gestation period of voles is 21 days. Up to eight young are produce at a time, the average number being about four per litter. Unlike white mic the vole will not rear its young in a nest containing any other families ; cons quently, when a female is found to be pregnant it has to be removed from t mating cage and placed by itself in a small cage containing a good surplus nesting material pushed up at one end of the cage. If after about 24 hou the female has made no effort to start building but has spread the hay all ov the cage, the hay must be scraped together again into a bundle and push back to one end of the cage. This does not often happen, but when it does a is rectified, the resulting nest is very shoddy and thin and the vole will probab eat the young when born. Even if an apparently good nest is being made watch must be kept to see that it is not too thin on top, for to rear a fami successfully the nest must be thick and dark inside. It does not matter if t nest is opened and the young handled or examined so long as the damage partially repaired and some more material thrown on top to cover the ho completely and make it dark again inside. In cases where it is necessary handle or examine the young several times a day, a nest-box about 4 in. 4 in. with a hinged lid and an entrance hole in one side is useful. This can stuffed full of hay and dry grass, and when the nest is made the top can removed so that only the wooden lid remains. When this is lifted up, ea access to the middle of the nest is immediately possible ; but for ordina breeding purposes this arrangement is quite unnecessary and only increas labour and expense.

In a small family of two or three the young can be weaned from an age 12 days onwards, but it is usually better to leave them a little longer, since ear weaning acts as a check on their growth. If weaned at 14 days their quic development is assured, and the parent female is given a little longer to recov condition. The young males will take 6 weeks to become sexual mature, but the young females become perforate in as short a time as 20 da from birth. No fertile matings took place in the laboratory in less than 30 da from birth, but from evidence collected during the dissection of 2,500 wi voles (Baker & Ranson, 1933), it was concluded that in the case of the femal fertile mating can take place about a week after leaving the nest, i.e. abo 21 days from birth.

In the wild state during the height of the breeding season the female h an œstrus period directly after the birth of the young and will copulate aga at once, so that a continual increase can occur every 21 days. It is not possib to breed at this rate in laboratory stock owing to the difficulty of knowi exactly when the young are born and of taking the female away without t young dying, for if a male is added to the nesting-cage it will destroy the ne and eat the young. The most successful method of weaning is to remove t female and leave the young in the nest for 3 or 4 days, when they can transferred to the growing-cages and kept in batches of 10 to 15. The pare female is put into one of the breeding-cages straight away. During t growing period it is essential that an excess of food always be present, especial coarse hay and rough grass.

Pregnancy can be determined at a very early stage by gently pinching t abdomen with the thumb against the first finger resting across the back, whe after a little experience both horns of the uterus and the rectum and the kidne can easily be felt. The embryos are most readily felt when they are abo

n. in diameter or of about 12 to 14 days development. I have diagnosed nancy at 5 days after conception by this method, although at that early the swellings are barely visible on dissection. Further details of preg- diagnosis are given by Ranson (1941).

5. Handling and Transport

oles cannot be handled with quite the same freedom as white mice with- ne risk of being bitten. They should be picked up firmly by the loose skin e back, in the region of the thorax, with the thumb and two fingers. If are to be held for a long time it is preferable to put them on the palm of and and move the hand up and down slowly. The movement will cause to have insufficient confidence to jump off and they will remain quite still. or transport we used the small cages roped together in tiers of four or five, six voles per cage ; but the whole of the cage was packed loosely with hay ich the voles could make runways and be out of the light. The hay also bs the shocks incidental to rail or road transport. A large excess of food in, and a board or sheet of thick brown paper is put over the lid of the age to prevent the unwelcome attention of travellers who might put in ous food. If the voles are to be more than 24 hours in transit the number age should be reduced to four and more food should be put in. Wild are commonly sent distances of over 300 miles by train, and will survive ut harm for at least 16 hours. We have little experience of sending voles distances, but would suggest large zinc-lined boxes with plenty of ation holes, peat moss at least 1 in. deep on the floor, and the whole box quarters filled with tightly packed hay. A close-fitting solid zinc-lined th a lock and hinges, and cabbage or lettuce leaves substituted for fresh would also be desirable.

r further details of the transport of live voles over long distances, see (1938).

6. Disease

teria of health. The growth-rate after weaning, results of breeding, ge duration of life, absence of excess fatness and the general appearance l-being (evinced by the state of the fur and the degree of activity) have en taken into consideration as criteria of maximum health.

e condition of the fur and eyes is the most reliable indicator. The fur l be glossy and smooth and lie sleekly from the crown of the head down the and the flanks to the tail ; the cheeks, chest and legs are often not so h. The eyes should be bright and should stand out from the head like

When a vole is in bad health the eyes will go dull and recede and the s may be partly stuck together with dry mucus ; the fur will look dull usty and will stand up on the crown of the head and forehead ; the back e arched as though the animal is trying to conserve heat by making itself erical as possible ; finally, the animal will lose weight rapidly.

le tuberculosis. This occurs under natural conditions. For details, see (1937), Griffith (1937, 1939) and the bibliographies by Elton (1942).

xoplasmosis. As indicated above, natural epizootics in voles have been ated with a protozoan infection of the brain due to *Toxoplasma*. This en successfully transmitted to stocks of normal healthy voles (Findlay & eton, 1934 ; Elton, Davis & Findlay, 1934).

Other parasites. The health and parasites of a wild mouse populatio including *Microtus*, were studied by Elton, Ford, Baker & Gardner (1931 The work of Davis on the fauna of voles' nests, and their fleas, and all oth work on naturally occurring epizootics of voles, is referred to in Elton's (194 monograph.

Mortality rate in laboratory stocks. This has been studied by Leslie Ranson (1940) and by Ranson (1941). Many losses among new-born voles ha been found to be due to "rogueness" in one of the parents. The criterion rogueness suggested by Ranson is for a vole to have absorbed or killed at bir most of the fœtuses in at least 50 per cent. of its litters. In point of fact it h been found that once a vole has shown rogue tendencies it hardly ever retur to normal breeding life, and usually if it is going to be a rogue it will start the first litter it produces. It is not known whether rogueness is a genet weakness or a vice which is acquired, but it is possible for either the male the female to be a rogue, and for a normal parent which has produced norm litters when mated to another normal to produce rogue litters when mated a rogue. Habitual litter-eating in rats and mice is referred to in Chapte 6 and 8 respectively.

Virus diseases (Experimental). Experimentally it has been shown thl voles are highly susceptible to Rift Valley fever (Findlay, 1932), louping (Findlay & Elton, 1933), climatic bubo (Findlay, 1933), yellow fever (Findla 1934) and foot-and-mouth disease (Edwards, 1937, p. 195). With the increa ing use of the vole as an experimental animal it might be anticipated that, with the mouse, enzootic or latent virus infections will come to light.

Note added in proof.—Mr. H. V. Thompson, in a letter dated 24 June, 194 adds the following information based upon recent experience at the Bureau Animal Population, Oxford :—

1. Up to two dozen recently weaned (4–6 weeks old) *Microtus agres* of mixed sexes have been kept in a 36 in. × 24 in. × 12 in. colony ca with excess hay and no fighting resulted.

2. Hay can be put in Topley cages quite safely, the risk of water sipho ing out of the water bottle being negligible.

3. Male voles can live quite happily together in the presence of femal (see 1).

4. Owing to a shortage of cages, I have recently allowed *M. agres* females to rear their families in the same nest, in the same cage as t parent male, with complete success.

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Note added in proof. Boden-Heimer, F. S., & Sulman, F., (1946). *Ecology.*, 27, 255–2 "The estrus cycle of *Microtus guentheri* D and A and its ecological implications Boden-Heimer & Sulman state that this species ovulates only as a response copulation. Although observations on wild British voles of various species have n so far given any indication that this is so in their case, the matter ought to be inv tigated under field and laboratory conditions.—*Ed.*

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CHAPTER 14

THE ORKNEY VOLE

(*Microtus orcadensis*)

[Compiled.]

1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Handling.

1. Introduction

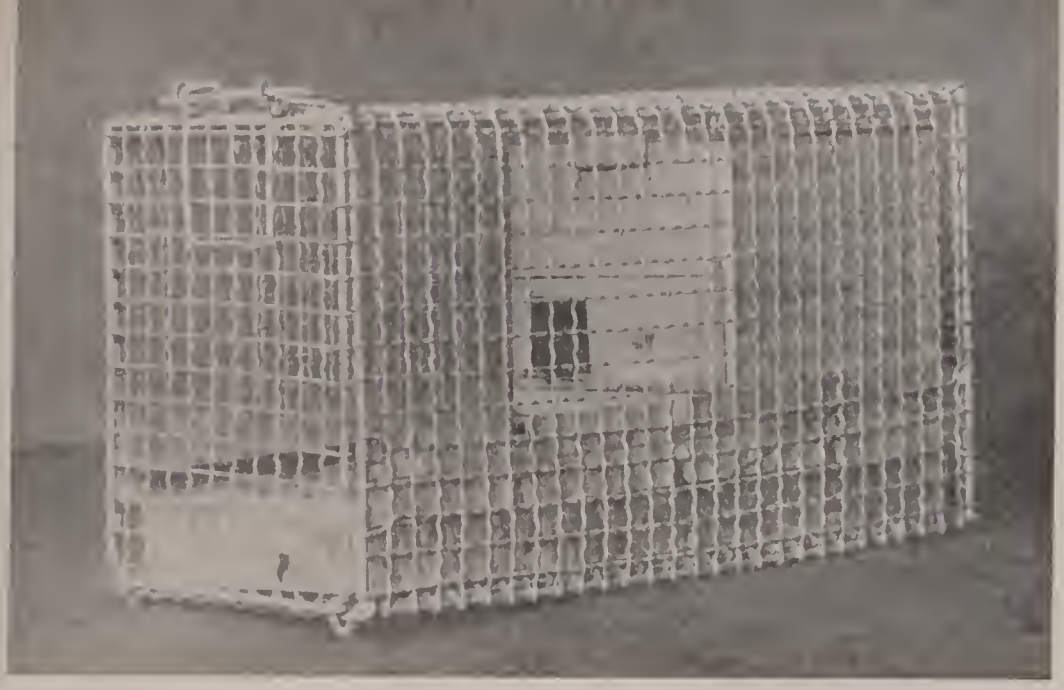
The Orkney Vole, *Microtus orcadensis*, is a larger species (117 mm., pl tail, 38 mm.) than *M. agrestis* and can swim well; sub-species include th Ronaldshay, Ronsay, Westray, and Sanday voles (Sandars, 1937). It ha two great advantages over *M. agrestis*: (1) it has a much longer life and th average expectation, although not yet completely worked out, is greatly excess of the 220 days found in *M. agrestis* in the laboratory; (2) it stands u much better to conditions prevailing in a pathologist's animal house (Elton 1943). It is susceptible to experimental infection with the vole strain *Mycobacterium tuberculosis*.

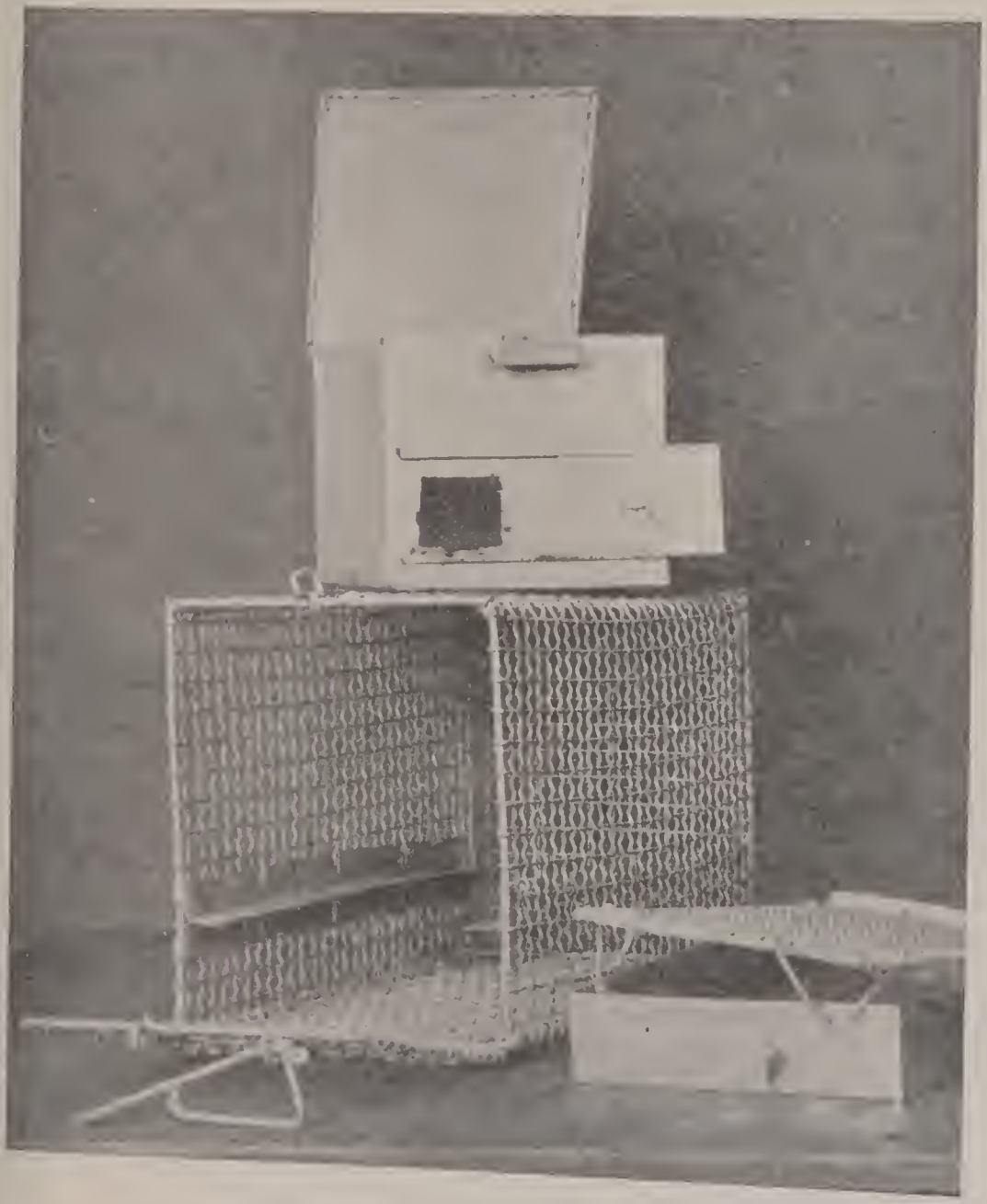
The Orkney vole was introduced into the Bureau of Animal Populatio Oxford, several years ago and has been maintained as a homogeneous stock with no new blood added, since then. Breeding colonies are maintained als at the Agricultural Research Council's Field Station, Compton, near Newbur Berkshire, and at the Farm Laboratories of the National Institute for Medic Research, Mill Hill.

In the information which follows only points which differ from those con cerning *M. agrestis* will be given. In other respects treatment should follo the course advocated for *M. agrestis*.

2. Accommodation

Thompson (1945) keeps these voles successfully in the metal cages, 12 in. 8 in. × 5 in., used for *M. agrestis* or in galvanized rat-cages measuring 12 i × 12 in. × 7½ in. Topley glass cages 9 in. in diameter were found to be qui unsuitable. Dr. J. A. Young, of the Institute of Animal Pathology, Universit of Cambridge, has designed the all-metal cage illustrated in Fig. 44. This cage, th outside measurements of which are 14½ in. × 6 in. × 8 in., is suitable for individu or paired voles, or for a female and young; it is suitable also for other sma rodents. The removable nest-box is fitted with a small entrance hole which ma be closed by a sliding door. It may, therefore, be used for removing the vol from the cage for transferring them to the inoculation room, and so on. Ca should be taken to mark the nesting-box as well as the cage. The animals c not foul their nests, which should contain suitable bedding material, e.g. pe or wood-wool. There is a sliding tray which should be filled with peat or woo chips, beneath the detachable grid floor.





44.—All-Metal Cage designed by Young for use with Orkney Voles. [*Photo: W. J. Smith.*
(The lower illustration shows the component parts.)

3. Nutrition

Dietary requirements. Kodicek & Worden (1942–44) have found that t Orkney vole does not thrive on a diet comprising sucrose 60 parts, light-wh casein 20, arachis oil 15, B.D.H. salt mixture 5 and dried brewer's yeast supplemented with vitamins A and D as Radiostoleum and vitamin E, althou this diet permits of rapid growth and normal reproduction in the laborat rat. Voles so fed lost their hair, became weak and emaciated, and if untrea died in from one to three weeks with a variety of pathological changes. addition to the ration of vitamin C or of inositol was without effect. addition to the diet of relatively small quantities of grass resulted in drama recoveries and permitted of normal growth and development. In a furt experiment it was confirmed that vitamins B_1, B_6, riboflavin, nicotinic ac pantothenic acid, choline, inositol and *p*-aminobenzoic acid (several of wh were supplied by Mr. A. L. Bacharach, Glaxo Laboratories, Greenford, Midd sex) did not replace the activity of the grass factor or factors.

That the vole is independent of vitamin C was confirmed by Kodicek Worden, who fed groups of voles on a scorbutogenic diet (oatmeal 72, bran dried egg-yolk 9, yeast 10, B.D.H. salt mixture 2 + vitamins A, D and with and without supplements of vitamin C. Growth and health in the gro not receiving vitamin C were fully as good as in those receiving the vitam Attempts are now being made to modify this type of diet so as to rende suitable for routine feeding. The use of such a dry diet would greatly les the labour involved in feeding voles.

Practical feeding. Thompson (1945) uses the following diet: a surplus hay and water; green vegetables and roots two to four times a week accord to season; about 10 g. whole oats per vole five times a week; about 10 g. wh wheat with 2 per cent. of cod-liver oil twice a week. Larger water-bottles the 120-ml. size) are used than for *M. agrestis*, since an adult Orkney vole n drink 50 ml. in a day. At Compton, Orkney voles are fed on hay, bread-a milk and vegetable mashes, and water.

4. Breeding

In the case of the Orkney vole there is no difficulty in breeding on colony system; indeed it gives better results than do individual mati (Glover, 1945). The main point is to start the colony with freshly wea animals and to allow them to interbreed as a unit. Under these conditi aggressiveness of the males is not encountered and several litters can be wea simultaneously.

Thompson (1945) breeds in pairs, quartets or harems and records the ge tion period as 21 days, with 1 to 6 (average 4) young per litter. The young weaned at 21 days of age.

5. Handling

Thompson states that Orkney voles if handled frequently from a few d old become almost as tame as white mice, but that if handled infrequently, once a fortnight, they become very wild and vicious and never relax w picked up but remain tense and trembling, and can inflict a very painful b usually at the base of the finger-nail.

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CHAPTER 15

THE GOLDEN HAMSTER

(*Mesocricetus auratus*)

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1. Introduction. 2. Characteristics of the species. 3. Accommodation. 4. Nutrition. 5. Breeding. 6. Anæsthesia and euthanasia. 7. Diseases.

1. Introduction

The *Cricetus* section of the subfamily Cricetinæ includes all the true hamster which are widely distributed throughout the temperate regions of Europe a Asia. Ten generic names have been given to these hamsters in recent years.

The best known forms are *Cricetus cricetus* (Linnæus), the ordinary Europe Hamster ; *Cricetulus griseus* (Milne Edwards), the Grey Hamster, which rang from Southern Europe to China, and *Mesocricetus auratus* (Waterhouse), t Golden Hamster, only known from Aleppo.

Various species have been used for infection experiments, especial *Cricetulus griseus* in China,* but the only species that has become establish as a laboratory animal is *Mesocricetus auratus*.

The Golden hamster is in many ways unique as a laboratory animal sin until recently only two examples were known, the type, a female from Alepp described by Waterhouse in 1839, and a second specimen found preserved the museum at Beirut. In 1930, I. Aharoni, of the Department of Zoolog Hebrew University, Jerusalem, dug up a burrow in the outskirts of Aleppo a found an adult female and 12 young ones. Mr. Aharoni was successful keeping these animals alive in captivity and took them back to Jerusale Since that date, in Palestine alone, more than 12,000 specimens have be produced from the original stock. The many thousands in Britain are all t descendants of two pairs presented to me by Dr. S. Adler in 1931. The stra was first maintained at the Wellcome Research Foundation, then at the Natio Institute for Medical Research, and subsequently at the Department of Zoolog University of Glasgow. These hamsters have now become widely distribut in laboratories in many parts of the world, but it is well to recall that they a all the descendants of one family found in 1930.

The term "Syrian Hamster," sometimes used to designate the Gold Hamster, is misleading and should be avoided since there are other species Syrian Hamsters.

2. Characteristics of the Species

The Golden Hamster is smaller than the common European Hamster full-grown female rarely exceeding a length of 7 in. It has a deep golden-bro

* For particulars of *Cricetulus griseus* in captivity, see Chang & Wu (1938). [For a Soviet ref ence to the use of the Transcaucasian hamster see Chapter 2. —*Ed.*].

olour, but towards the roots the hairs are dark grey. The fur is short, soft nd smooth. The ventral surface is very light grey, with white patches. The rs are large, grey and almost naked, with a few golden-brown hairs on the ıter surface. The eyes are large and black. The skin is extremely loose, to ch an extent that folds at least 2 in. deep can be pulled out from any part of e trunk. The short stumpy tail, and especially the feet, are lighter in colour an the rest of the body. The cheek-pouches are well developed and can hold surprisingly large amount of food. According to B. Aharoni (1932), these mmsters live in deep burrows which they make in grain-fields. Although in ture they are presumably herbivorous, in captivity they are omnivorous, d, in addition to grain, will feed on roots, nuts, bread, meat, etc., and will rry off and store almost any portable object.

This species in captivity soon becomes tame, and, unlike most hamsters, n be easily handled, especially if accustomed to it when young. Occasionally e meets a nervous individual, but so long as the animals are handled gently d are well awake before being picked up, there is little risk of being bitten. hen a sleeping hamster is suddenly disturbed it is liable to bite, but this is nerally the result of fright.

The hoarding instinct is evidently a strong psychological urge, and when ced in a new cage a hamster at once begins to form a food hoard. This stinct is more strongly developed in the female than in the male, and is present a very early age, since young only two or three weeks old may be observed ing their pouches. Although essentially nocturnal in habit, they come out their nests at all times of the day, but are not inquisitive by nature, and d to remain quiet for the first few seconds after being disturbed.

When hamsters are reared together in the same cage they do not as a rule m one another, but if one of their number is removed for some time and en returned to the original cage he is liable to be attacked and bitten. cording to Ben-Menahem (1944), not all the animals are the aggressors, and attendant will do well to single out the offender. If a wounded animal is nd in the cage its removal offers no true solution of the problem as the known aggressor will still be at large in the cage to seek and find another tim. It is worth spending some time finding the culprit, who should then removed. The wounded animal, when given the necessary treatment, may left in the original cage.

3. Accommodation

Hamsters are better kept in special rooms, not in those used for other oratory rodents, and the precautions regarding pests (Chapter 3) should be ngently observed. The optimum environmental temperature-range is to 22° C. and the optimum humidity 40 to 60 per cent. ; the room should iry but free from draughts.

Any conveniently sized cage may be used, but an all-metal type is preferable e hamsters are able to eat through wooden cages. The ordinary rat-cage suring 15 in. × 12 in. × 6 in. may be used for individual or paired hamsters. en breeding it is advisable, though not essential, to provide a dark chamber, er by means of a piece of bent metal sheeting, or by simply darkening part he cage with any convenient covering. The vole-cage designed by J. A. ng (Chapter 14), if slightly enlarged, would seem to be quite suitable for

hamsters, but personally I have had no difficulty in breeding them in what ever type of cage was available. The animals should be provided wit plenty of hay, or other bedding material, and the floor of the cage or tra covered with peat.

The stock animals may be kept in any convenient receptacle but the sexe should be kept separate. Large aquarium jars containing a few inches of san are excellent for keeping animals in good condition. Hamsters generall select some particular corner for the purpose of voiding excreta and if the san in this region is removed periodically the remainder of the sand will remai clean for a considerable period. If a number of hamsters of the same sex ar placed together in a large cage, or other container, they generally live togethe quite amicably ; but when any new individuals are introduced it is advisabl to take all the animals out of the cage, put in plenty of food, and then retur them together with the new-comers. When they get back to their cage th hamsters will at first be fully occupied in filling their cheek-pouches and makin new stores.

Their habit of voiding excreta in the same place has been used by Byl (1944) to devise a simple method of collecting urine. An opening 3 cm. i diameter is cut out of the bottom of the cage. Under this hole is placed glass receptacle and the hole covered with wire gauze stained with urin Experience shows that hamsters habitually urinate over the stained gauze.

4. Nutrition

The specific nutritional requirements of the Golden Hamster have bee studied by Prouth & Houchin (1942), Cooperman, Waisman & Elvehje (1943) and Hamilton & Hogan (1944). Prouth & Houchin reported tha nicotinic acid was a dietary essential, but did not include biotin in the diet Cooperman *et al.* found that young hamsters failed to thrive on a diet consistin of sucrose 72, casein 18, salt mixture (Phillips & Hart plus 5 g./kg. of magne sium sulphate) 5, maize oil 2, cod-liver oil 2, and wheat-germ oil 1, fed a *libitum*, together with one drop of halibut-liver oil every two weeks and dail supplements of 500 mg. each of vitamin B_1, calcium pantothenote, riboflavi and nicotinic acid, and of 1 mg. each of sodium *p*-aminobenzoate, inositol an choline. Over a four-week period the average growth of males was onl 0·66 g., that of females only 0·95 g., and there were many deaths. Survivo developed a dermatitis at the angles of the lips, especially if life was prolonge for more than six weeks. The addition of 3 per cent. of a 1/20 liver extra prolonged life but gave an insignificant growth-response. The addition 1 mg. of biotin daily resulted in good growth and prevented the appearance dermatitis ; the addition of 0·5 mg. daily was inadequate. Ascorbic acid w found to be unnecessary, a finding confirmed by Clausen & Clark (1943 Subsequent experiments suggested that nicotinic acid, inositol and *p*-amin benzoic acid were also unnecessary. Hamilton & Hogan (1944), likewise fou that nicotinic acid, inositol, *p*-aminobenzoate and also choline were unnece sary ; they found that young hamsters grew at a normal rate and attained f maturity on a basal diet of casein cerelose, lard, cellulose, salt mixture a vitamins A, B_1, B_6, D, E and K. (The casein and cerelose contained traces biotin.) Withdrawal of vitamin E from the diet led to collapse and death from 4 to 18 weeks, but recovery could be brought about by injection of 5 m

opherol, the effect in six out of seven animals so treated being dramatic. nen vitamin K was withheld growth was somewhat irregular. Animals ich died after being deprived of both vitamins E and K had hæmorrhagic ions. (Hamilton & Hogan's paper contains also a brief description of the itine management of the colony.)

Further observations upon the specific dietary requirements of the hamster l probably result from its use in the experimental study of caries (Arnold, 12).

Routine feeding. Bruce & Hindle (1934) used a regular diet (fed once ly) consisting of puppy biscuits, monkey nuts and mixed grain (wheat, s, barley, etc.) to which were added 2 or 3 g. per head daily of raw carrot, enstuff or sprouting tares, and fresh cow's-milk to drink. They found it icult to estimate food intake accurately owing to storing habits and to stage, but on a few individuals it was calculated that the daily intake was o 3 g. dry food per head and as much milk as they would drink. Small kly supplements of A, B and D were added during the winter months. ring the war this colony has been maintained on a diet of proprietary rat es, carrots and any green food available, together with a little milk when ainable.) The diet was adequate for breeding.

Ben-Menahem (1944) described similar methods and recommends the owing food ration per head from the fourth week of life : 14 g. of greenstuffs ed according to season and including carrots, 4 to 5 g. of grains, 2 to 3 g. nilk. If there is a shortage of greenstuffs, the ration should be supple- ted with a few drops of fish oil. Like other rodents, the hamster relishes t, but this is not essential for development and is undesirable, as hamsters stomed to eat meat may become cannibalistic, eating each other as well heir offspring.

At the Farm Laboratories of the National Institute for Medical Research, don, routine feeding is planned as follows : Monday, Wednesday, Friday Saturday, mixture No. 1 ; Tuesday, Thursday and Sunday, raw green food .) and monkey nuts (1 lb.) Mixture No. 1 consists of :

1 lb. 5 oz. Rangoon beans	} Sufficient for 150 hamsters.
13 oz. wheat	
13 oz. houndmeal	
2 lb. 3 oz. bread	
9 oz. gre[illegible]od, raw	
5,0[illegible]ize meal	

½ teaspoonful Marmite, replaced by 3 oz. cod-liver oil on Wednesdays.

goon beans are soaked overnight and steamed for 2½ hours. Houndmeal is ed for 4 hours and the bread for 2½ hours. Beans, green food and wheat minced together and then mixed with bread and houndmeal and dried off maize meal. Marmite is added except on Wednesday, when cod-liver oil .) is substituted. All mixing is done by hand, and the diet is fed imme- ly it is prepared. No food must be given that has been prepared for a r period than six hours.

ne pint of tares, soaked overnight in cold water, is fed daily.

ix lb. of sprouted grain (3 lb. oats and 2 lb. wheat) is fed every other nesday in place of green food.

uring the winter months, when green food is scarce, raw mangolds are

fed instead. During May and June grass may be given as an alternative green food.

5. Breeding

The reproduction of the golden hamster has been studied by Bruce Hindle (1934), Deanesly (1938) and Ben-Menahem (1944). In additic Peczenik (1942) has investigated the action of sex-hormones on the œstr cycle and reproduction.

Hamsters generally mature at the age of 10 to 15 weeks, males being som what more rapid in development than females. Bruce & Hindle (19 record a litter born from a brother-sister mating when they were only 68 da old ; allowing for a minimum gestation period of 16 days, in this case fertili tion must have occurred when both parents were only 52 days old. In m cases, however, the female does not mature before 12 weeks old and it advisable not to breed until they are at least 15 weeks old.

For breeding purposes I have always used cages containing one male a one female and removed the male after 12 to 14 days. Ben-Menahem (19 puts one male with three females and after 10 days observes which are pregn by means of palpation. These are removed to individual cages supplied w plentiful bedding for nest-building. The ration is improved by the addit of germinated wheat, and water or milk should be provided. After parturit the cage should be disturbed as little as possible, otherwise the mother is lia to neglect or injure the young.

Under laboratory conditions approximately one-third of the females in stock have failed to breed, although given every opportunity of mati Peczenik (1942) has studied this phenomenon and found that such individu as well as females that have ceased breeding as a result of old age, respond to injection of œstrogens and may become fertile. Moreover by this means, litt may be obtained throughout the year, although usually breeding is suspen from November to March.

Presumably copulation takes place during the night for we have ne observed it during the daytime. The male leaves a plug in the vagina of female, which can be found by examination and gives an indication of exact date of impregnation. The gestation period is unusually short fo mammal of this size, averaging only 16 days, although occasionally it m extend a day or two longer. The number of young in a litter varies c siderably, ranging from 3 or 4 in the first litter up to 12 or [illegible] later litt the average being about 7 per litter. Mothers sometimes kill you[illegible] exc of this number and it may be advisable to remove some of the young when number is excessive.

The young are naked when born and very small, weighing about 2 g. ea Hair first appears after 5 days and the whole body is covered after 8 da After about 11 days the eyes begin to open and the hamster then weighs ab 9 g. Subsequent development is rapid and the young starts to feed itsel addition to sucking from the mother. The hair which first appears is dark brindled on the back, but the coat soon becomes tawny and after 3 or 4 we shows the typical golden-brown colour of the adult. The young can then weaned.

In my experience females rarely produce more than three litters and a rule cease to breed when about nine months old, although both sexes may l

r at least two years. Unusually heavy females are poor breeders and none our individuals exceeding 180 g. in weight has been fertile. On the other and, pregnancies have occurred in females weighing only 75 g.

After breeding ceases there is usually a marked increase in weight, some males reaching as much as 250 g.

6. Anæsthesia and Euthanasia

Etherization, as recommended for rats (Chapter 6), is suitable for anæsetizing hamsters. For narcotic purposes Patton & Hindle (1927) found minal effective ; repeated injections seemed to have no ill-effect.

No special method of euthanasia has been described ; the methods suggested r the rat, Chapter 6, would seem to be suitable.

7. Diseases

Bruce & Hindle (1934) note that, despite the peculiar susceptibility of the mster to *Leishmania* infection, it appears to be relatively free from natural ections.

Notoëdric mange is the most common infestation, as hamsters seem to be rticularly susceptible to the burrowing mite, *Notoëdres notoëdres*, and the ection may spread from the ears to other parts of the body and cause much bbing. Sections of infected ears show the presence of numerous mites, væ and eggs. The infection responds to treatment with the usual antinge remedies, but two recent I.C.I. products are by far the most effective. th are organic sulphur compounds : the first, dimethyl diphenylene disulde, an oily substance known as " Mitigal," and the second, tetraethyluram monosulphide, put up as an emulsion of 25 per cent. strength. Each these compounds is supplied in 10 per cent. emulsion in commercial coconut

The affected areas are rubbed with the emulsion every two or three days l four treatments are usually sufficient to effect a complete cure (Fulton, 3). I have used the second of these compounds, supplied under the name " Tetmosol," and invariably obtained cure even in the most severe cases. reover stimulation of the growth of hair around the affected areas was iced.

In common with most rodents hamsters are susceptible to **paratyphoid** and n outbreak occurs it is most important to take strict hygienic precautions deal with it. The symptoms include diarrhœa, lack of appetite, ruffled r, etc. Ben-Menahem (1944) also reports the occurrence of an infectious umonia, causing catarrh, emaciation and lack of appetite. The only nedy is to destroy affected and contact animals. The same author also cribes an obscure but fatal infection known as " **wet-tail** " **disease**.

I have not met with any of these infections, but as a rule the stock was kept rt from other rodents that might have been responsible for the introduction xtraneous diseases. On one occasion several deaths occurred within a few s from onset as the result of an **obscure disease of young stock.** Loss of ght was the only obvious condition ; *post-mortem* examination did not eal any significant lesions and both aerobic and anaerobic cultures were ile.

Wounds resulting from bites may become infected and should be cleane and dressed or they may lead to subcutaneous lesions and pyæmia.

Various pathological conditions have been met with occasionally in my stoc including **melanomata,** not infrequent in older animals, **middle-ear disease** in single male, and **microphthalmia,** transmitted as a Mendelian recessiv character. *Bartonella and Eperythrozoon* infections were not found in nin hamsters examined for these organisms, although they could readily be infecte with strains recovered from rats and mice.

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CHAPTER 16

THE FERRET

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1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Handling. 6. Diseases.

1. Introduction

In the last twelve years the ferret has come into increasing use as a laboratory animal. Although practical breeders have long been aware of this animal's susceptibility to canine distemper, it was not until Dunkin & Laidlaw's (1926) studies on the disease, in which they employed the ferret, that the attention of laboratory workers was drawn to its possible uses as an experimental animal. Since that date the ferret has been used in the investigation of a number of virus infections, including the well-known work in this country (Andrewes & Glover, 1941 ; Glover, 1941), and in the United States of America the influenza viruses.

Providing precautions are taken to avoid the accidental introduction of these infections, the husbandry of the ferret presents few difficulties. As with other laboratory animals, it is preferable for a laboratory to rear its own stock of ferrets in a self-contained unit. When the numbers used are insufficient to justify such an undertaking, ferrets should be obtained from a breeder who understands the importance of rearing them free of disease. Newly purchased ferrets should be kept in isolation for at least 10 days to a fortnight before they are used, and an additional check on their health may be made by taking temperature records.

2. Accommodation

The standard cage is of the hutch type, divided into an exercising and sleeping run open to view, and a dark secluded sleeping compartment. Although the ferret is an unusually clean animal, its body odour and droppings are offensive and constant attention to hygiene is necessary if the ferret quarters are not to become noisome. This entails having the cages made of materials capable of withstanding frequent washing and immersion in water. At these laboratories the cages are made of wood, the interiors being lined with asbestos. Asbestos is resistant to water and it is warmer than metal. These hutches are 3 ft. 10 in. long × 1 ft. 6 in. wide × 1 ft. 4 in. high internally and are divided into two equal parts by an asbestos partition, with a pophole 4 in. in diameter in the centre, raised 1 in. above the floor-level. This hole can be closed by a sliding door. Each compartment has a door, that on the exercising run being composed of vertical metal bars $\frac{3}{4}$ in. apart, that on the sleeping compartment of wood, with a small perforated zinc grating let into it for ventilation. [Further information on ferret-cages is given in Chapter 2.—*Ed.*]

It is also possible to keep ferrets in courts with a number of sleeping-boxes

opening out on to the court. Such a system is described by Carnegie (192 The open court should be lined and floored with tile or concrete. The sleepin boxes should have the usual 4-in. pophole facing the court, and some form trap at the back to facilitate cleaning out the boxes. This system is best suit for housing numbers of young adult ferrets before they are mated and for kee ing ferrets for issue. The sexes must be kept separate under such conditio

[Glover (1945) has found the colony system successful. Males and fema in the proportion of 1 male to 5 females are allowed the run of a room with asbestos floor. Sawdust is used as bedding. The pregnant females a removed to hutches. As soon as young ferrets are weaned they are separat from the mother and formed into a fresh colony. Under these conditions t males and females become accustomed to one another. No injuries caused fighting have been encountered when this system is adopted.—*Ed.*]

The accommodation for experimental ferrets may have to be more elabora especially if the animals are inoculated with some of the more infectic disease agents. Horsfall (1940) has illustrated the ferret quarters used by h in his studies on influenza. I have been unable to trace the article promised him, in which these quarters are described. However, it appears that ea animal is confined in an airtight metal cage through which filtered air is dra by suction. Each cage is connected to the ventilating system by a pipe whi can be disconnected so that the cage can be removed without being opene Dunkin & Laidlaw (1926) used modified Topley cages made of sheet zi cubical in shape and measuring 18 in. each way. The lid, perforated ventilation, also had two holes 2 in. in diameter for the introduction of foo One of the sides of the cage had a glass plate 8 in. square, for inspection purpos Near the bottom of the cage there were two tubular holes 4 in. in diamet closed by sliding plates. Cages placed in apposition formed an inter-commu cating tunnel by which the ferret could be induced to change cages. The wh object of the design of these cages was that the ferrets could be observed a cared for without handling. [Andrewes (1945) suggests, however, that th refinements used by Topley for his mouse work are unnecessary for fer work, and that a system of isolation cubicles is better and more practica than Horsfall's system for work on distemper and influenza.—*Ed.*]

Bedding should comprise grass hay of soft quality and the cage floors sho be well covered with sawdust or peat-moss litter.

3. Nutrition

The dietary requirements of these animals are simple. Opinions vary to whether meat is essential, but amongst practical ferret-keepers it seems be agreed that a diet of raw meat keeps the animals in good condition. Ab 4 oz. of raw horse meat a day per head should be fed. Milk diluted 50 per ce with water should also be supplied. Ferrets may be kept on diets of bread a milk, whole wheat flour and milk, or oatmeal and milk, but these latter fo tend to cause a certain looseness of the bowels and watery and offensive dr pings. Feeding should take place once daily at a fixed time. The you while still in the nest may be gradually weaned on to a diet of bread soaked milk with finely chopped raw meat. The jill during the suckling period sho have ample supplies of whole milk. At first the young ferrets should be thrice daily, the feeding being gradually reduced to one meal a day.

eeks the young are beginning to find their way about, and at 2 months they y be removed from their mother.

[Little is known concerning the specific dietary factors required by the et. Kodicek & Worden (1943) have obtained preliminary evidence that otinic acid is essential.—*Ed.*]

4. Breeding

Numerous observations have been made on the breeding habits of ferrets. s literature has been summarized by Murray (1941) in his article on the eding of ferrets in South Africa.

In the northern hemisphere, the female ferret comes into season in early ch of the year following birth. Œstrus occurs again in July if the ferret been bred at her first season. There is great enlargement of the vulva ng œstrus. Hammond & Marshall (1930) state that, in the absence of us, œstrus will continue up to 5 months and that such enforced abstinence es considerable emaciation and weakness and may result in death. [This not been the experience with the Medical Research Council stocks at Mill where females have been kept for up to 12 months without ill-effect. *d.*]

The act of mating takes a variable time, from 15 minutes to 3 hours, with verage duration of 2 hours. Ovulation occurs at any time during œstrus, t takes place only after coitus.

The period of gestation is 42 days. The average size of the litter is 6 to 8, litters containing 10 to 12 young are not unknown. False pregnancy occasionally be met with. The young when born are hairless and blind. r weight at this time is approximately 10 g. Their eyes open at 4 weeks of nd at this time they begin to eat solid food.

s the time of parturition approaches, the pregnant female must be supplied ample bedding material. This material is used to make a nest which the r female ferret lines with her hair. When the litter is born the mother covers the young with a canopy of bedding. The mother and litter should sturbed as little as possible for the next 2 or 3 weeks, and the litter should xamined only when the mother is feeding in the other half of the hutch. ts are apt to destroy their young if the latter are interfered with.

Minett & Sreenivasan (1944) report the successful breeding of ferrets at eswar, in the Himalayan foot-hills, at a height of 7,500 ft.—*Ed.*]

5. Handling

errets can inflict nasty bites and the less used they are to handling the prone they are to cause such injuries. It is, therefore, necessary to tom them to frequent handling as soon as possible. Most ferrets become nably docile with constant handling. Normally they should be gripped l the neck with the fingers encircling the throat and shoulders. Further int such as light ether anæsthesia may be necessary for certain inocula- and other procedures.

6. Diseases

stemper. This is the most serious disease of the ferret. It causes a high lity and can wipe out entire stocks. Dunkin & Laidlaw (1926) have a classic account of the disease in this animal. The incubation period is

between 10 and 12 days. There is congestion and watery discharge from eyes and nose, and these discharges rapidly become purulent. There is ves formation on the chin, and the skin of the feet and abdomen is injected. animals which survive for four days or longer nervous symptoms may m their appearance. These commence as muscular tremors and twitchings wl may be followed by violent generalized convulsions and screaming. Owin the infectious nature of the disease treatment should not be attemp Affected ferrets should be killed and a vigorous policy of disinfection and is tion pursued.

Every attempt should be made to exclude the possibility of introduc such viruses as distemper and **influenza** into the ferret quarters. The at dants should wear waterproof coats and boots, which they should wash d with disinfectant solution before entering the ferret-house. Disinfect troughs to walk through should be provided at the doors, and the doors sh be kept locked. Visitors to the ferret quarters should not be encouraged.

Foot-rot. This is another common condition in ferrets. It is caused b mange mite. Insanitary conditions in the hutches appear to favour the co tion. The feet become swollen and covered with scabs, particularly in interdigital spaces. If allowed to progress the disease may cause loss of claws. Treatment when undertaken early is successful. The feet should soaked in warm water to soften the scabs, which are then removed. Iod or kerosene can then be applied topically to the affected parts. Carnoch quoted by Pyle (1940), advocates the use of xylol with a 3-day interval betw applications, followed by Danish ointment.

Scabies. Scabies affecting the back and tail also occurs. Treatm similar to that used for foot-rot, sulphur ointment, and benzyl benzoate are suitable.

Abscesses. Abscesses of the neck region caused by *Staphylococcus au* are recognized as a disease of these animals. These lesions may involve salivary glands, and in one case in my experience caused erosion of the bo of the base of the skull. The method of infection is not known.

Streptococcal pneumonia. Pneumonia from which hæmolytic streptoc can be isolated may cause sporadic losses. Brightman, quoted by Pyle (19 has described the isolation of group C streptococci from the lungs of fer inoculated with bacteriologically sterile influenza material. In these labo tories I have had cases of naturally occurring empyema from which I obtai group C streptococci.

[*Editorial Note.*—Glover (1945) notes that ferrets are very susceptible to **tubercul** Animals fed on unboiled milk may acquire severe alimentary tuberculosis with subsequ generalization. Unless milk from tuberculin-tested cattle is available, some form of h treatment is desirable. Spanneda (1941) has described **paratuberculosis** and Cout (1932) natural generalized **toxoplasmosis** (with *T. laidlawi*). An **epizootic disease of fe** with a very high case fatality rate has been described by Slanetz & Smetana (19 who found no immunological relationship between the causal filterable virus and viruses of canine distemper and of human influenza. Secondary invasion of the resp tory tract, mainly by hæmolytic streptococci, was common.]

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CHAPTER 17

THE HEDGEHOG

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1. Introduction. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Hibernation. 6. Handling. 7. Anæsthesia and euthanasia. 8. Diseases.

1. Introduction

This chapter was originally to have been drafted by the late R. M. Ranso of the Bureau of Animal Population, Oxford, on the basis of experience bo at Oxford and at Pirbright. His lamented death made this impossible, althou his observations up to 1941 have fortunately been summarized in his pap published that year in the *Journal of Hygiene*.

At Oxford a system of breeding hedgehogs was worked out by Ranson, a is referred to in Section 4 below. At Pirbright all the hedgehogs employ were obtained from the field, and the following summarizes the experien gained in working with large numbers of them over several years. Hedgeho are caught during the normal active (non-hibernating) season—March or Ap to October or November,* and from the moment of capture are kept sing i.e. apart from each other, for at first they tend to bite each other viciously the feet, and fatal suppurative lesions arise from the bites. Our dealers h special boxes made with small compartments into each of which a captur hedgehog was placed. On arrival at the laboratory care should again be tak to keep the hedgehogs separately until they are acclimatized, and a caref inspection made of each at once for injuries, blemishes, etc. None exce those which appear to be fit is worth retaining in the normal stock.

For some time after arrival at the laboratory hedgehogs may refuse fo and steadily lose weight. For experimental work it is essential that the hedg hogs should be known to be feeding well and gaining weight or, at any ra not losing weight. The refusal to eat is more noticeable in older hedgeho than in younger ones, and in some seasons the fresh arrivals, for some unkno reason, are more prone to refuse food than in other seasons. Although hedg hogs are omnivorous and can thrive on a large variety of food, such as ta scraps, bread-and-milk, raw meat, liver or kidneys and other offal, they a sensitive to the quality of the diet during the early part of their captivit Hence particular attention needs to be paid to the appetite and feeding of ea individual.

For detailed records of many matters relating to the hedgehog, see t monograph of Herter (1938), and for the more important recent work sin then on metabolism, see the several articles by Suomalainen. A brief revi of the literature was given by Hulse & Edwards (1937). Hedgehogs ha

* C. W. Hume suggests that on humane grounds the months during which the young nursed (May, June, August, September) should be avoided.—*Ed.*

en used for several years, largely on account of their seasonal hibernating cle, in fundamental physiological researches upon heat regulation and changes the endocrine system concurrent with the hibernating and sexual cycles, ich are very marked in both males and females, and for work in certain ectious diseases.

2. Accommodation

The rooms in which the hedgehogs are kept, as normal stock or for experi-nt, should be maintained at a steady temperature of 65° F., summer and ıter, and great care should be taken to avoid draughts and proximity to cold-shed stone floors, which may induce hibernation. In our work, in which cial pains had to be taken to prevent the onset of hibernation, the rectal nperature was taken regularly with an ordinary vaselined thermometer (not linical thermometer). Any that showed a sharp drop below the normal lgehog temperature of 95° to 98° F. were suspected of commencing hiberna-n and steps were taken to change their cages to a warmer place to prevent it. For work on a large scale we have partitioned wooden boxes made for ısing our normal stock, as shown in Figs. 45 and 46. Each hedgehog can

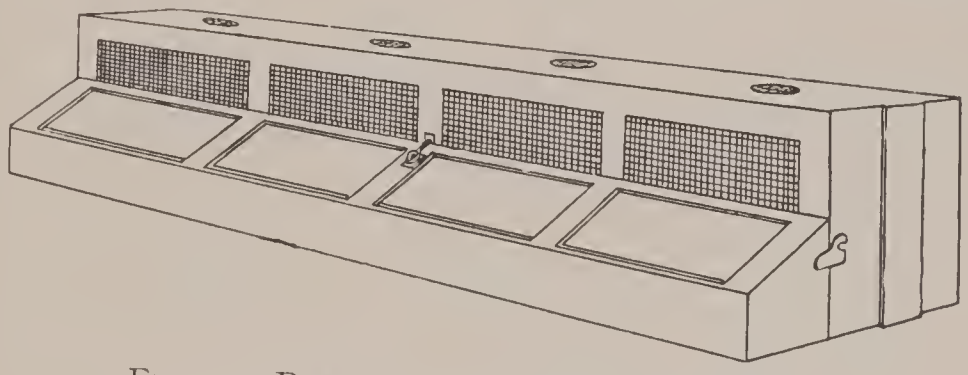


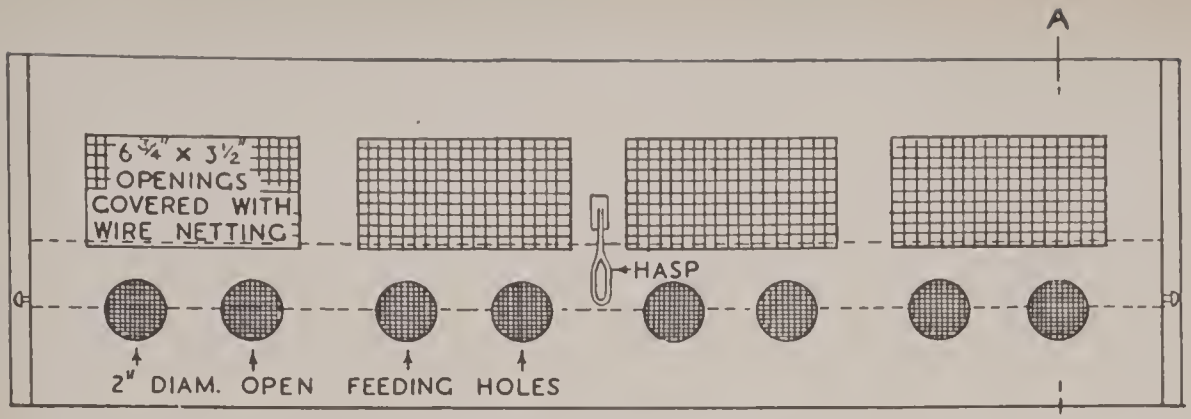
FIG. 45.—BOX FOR NORMAL STOCK HEDGEHOGS.
(Foot-and-Mouth Disease Research Station.)

fed separately, through holes in the front of its compartment, from the ıes placed in the detachable front of the cage. The greatest economy in ce is effected in this way, for a large number of these houses can be placed shelves fixed on the wall of the animal room.

Attached to the front of each normal hedgehog compartment is a *normal gehog history* card, on which are entered, once weekly, particulars regarding hedgehog's weight and feeding (feeding well^{+++}, feeding moderately^{++}, ing a little^{+}, or not feeding ^{0}). During the first few weeks the hedgehog be off its feed, partly or entirely ; but usually comes on to its diet gradually, steadily regains weight. Often, however, the hedgehogs may take kindly heir diet from the first.

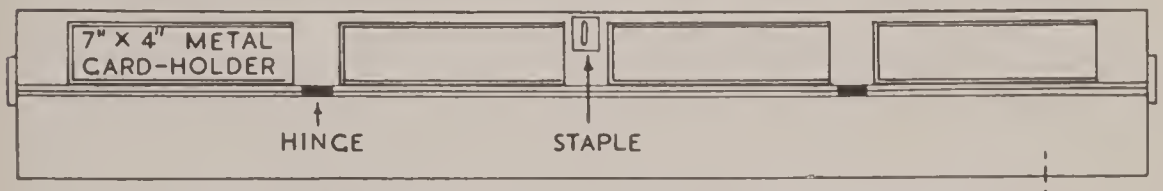
As both males and females in this species have a very well marked rutting on, and the males are much more pugnacious at this time than in the non-ing season, care should be taken not to put together specimens that show cially strong worrying and pugnacious tendencies.

While under experiment the hedgehogs are kept in small cylindrical metal , except in contact experiments, when special runs and compounds are structed. They are bedded down on hay or sawdust.



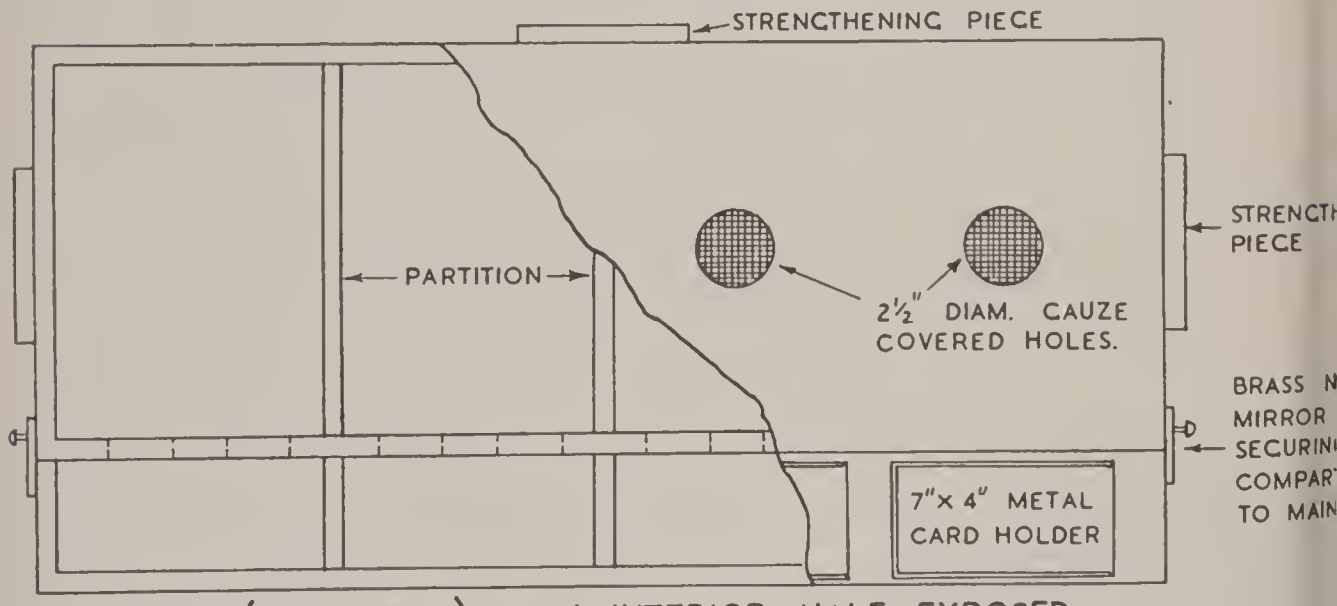
FRONT ELEVATION

(Dotted lines show position of food compartment when attached)

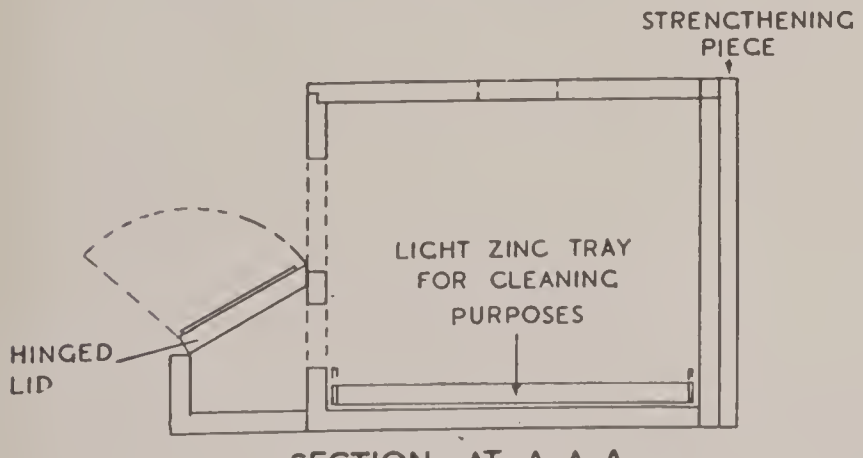


FRONT ELEVATION OF FOOD COMPARTMENT

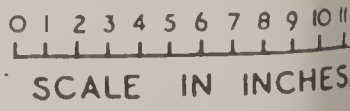
(Shown detached.)



PLAN (FROM TOP) WITH INTERIOR HALF EXPOSED



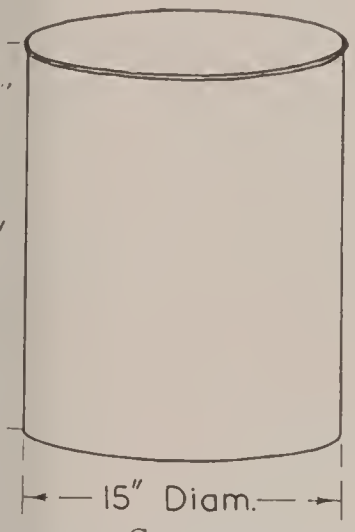
SECTION AT A-A-A



SCALE IN INCHES

FIG. 46.—STRUCTURAL DETAILS OF HEDGEHOG BOX ILLUSTRATED IN FIG. 45.

The breeding-pens and breeding-cages used by Ranson at Oxford (Fig. 48) described by him (1941) as follows : " The breeding pens used were 12 ft. uare, 5 ft. high, and covered with 1-in.-mesh wire netting, figure [48]. The e netting was buried 10 in. in the ground to guard against burrowing. The of the pen was covered with the same kind of netting. These pens were ginally designed for keeping partridges and are unnecessarily large. The atest density at which breeding occurred was one animal to 24 sq. ft. Some aller pens have been designed in collaboration with Mr. J. Francis, and made him at the Cattle Testing Station at Pirbright. These have so far proved y satisfactory for keeping hedgehogs, although no breeding has occurred re yet. They consist of a series of seven pens, two 9 ft. × 10 ft. and five t. × 5 ft. The sides, which are of $\frac{1}{4}$-in.-mesh wire netting, are 2 ft. 6 in.



G. 47.—GALVANIZED-IRON USED FOR INDIVIDUAL EX-MENTAL HEDGEHOGS AT FOOT-AND-MOUTH DISEASE CARCH STATION.

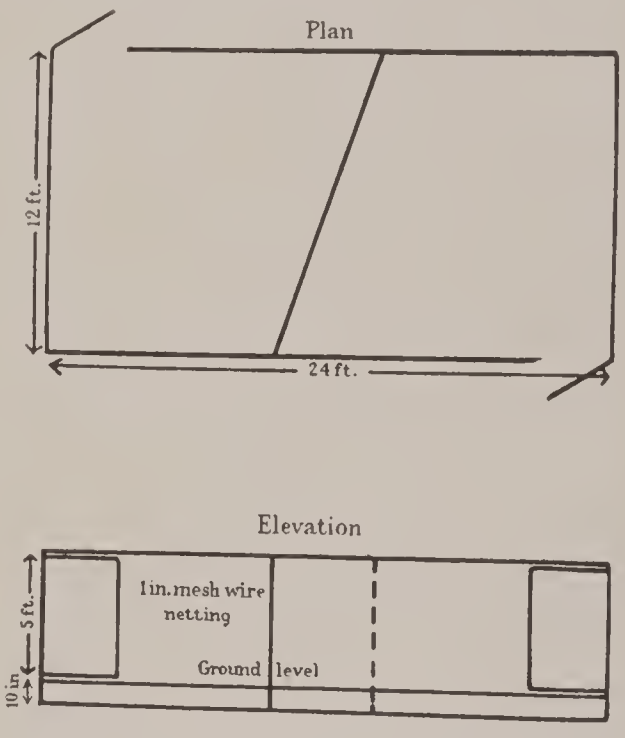


FIG. 48.—PARTRIDGE BREEDING-PENS USED FOR HEDGEHOG BREEDING AT OXFORD.

(Reproduced by courtesy of the Editor of the *Journal of Hygiene*.)

from the ground, and 1 ft. 6 in. is buried to prevent burrowing. A baffle-d runs round the top of the netting, projecting inwards 9 in. to prevent the ehogs from climbing over (they can climb over 6 ft. wire netting in a few nds). The board covering the common side is 14 in. wide, allowing a pro-on of 7 in. into each cage. The tops of the cages are uncovered.

A small shelter or a nest-box is provided in each pen and this is stuffed with hay or straw. The large pens can accommodate a harem of four, the small ones are large enough for a pair.

When the females are pregnant they can be put into much smaller cages, × 2 ft. and 1 ft. high. These can be either metal or wooden bins with and with wire netting or wooden framework lids, or wire netting on a en frame with a netting floor and a 1-ft. × 1-ft. nest-box attached to one These framework cages are kept out-of-doors on grass and are moved the ground under them becomes foul.

" If the pregnant females are brought into an animal house and kept bins, the floors of the bins should be covered with sawdust or granulated pea moss. A heap of dry hay or straw at least ten times the volume of the anim should be put at one end as nesting material. Two jars should be provided each cage, one to contain food and the other water."

3. Nutrition

For feeding our large stocks of hedgehogs the following dietary was work out :

	lb.	oz.
(1) Meat offals : 19 lb. 6 oz. : as follows—		
Tripe (undressed)	3	6
Breast of mutton	3	6
Flank or skirt	3	6
Lights, lung or spleen	3	6
Suet	1	6
Liver (*raw*)	4	8
(2) Root vegetables (i.e. potato, carrot or swede)	18	0
(3) Biscuit (wheaten wholemeal)	9	0

(Weigh when dry, and soak in 11½ pints of water.)

All the above ingredients, with the exception of liver (which must be *raw*) should be steamed together until cooked. Then they should be minc together with the raw liver.

Add to the minced mixture :

	oz.
(4) Cod-liver oil	1
(5) Dried yeast	1
(6) Bone meal	
(7) Salt mixture	

The stock salt mixture is made up as follows :

	g.
Calcium carbonate (precipitated chalk)	1,400
Sodium chloride (common salt)	350
Potassium chloride	50
Magnesium sulphate (Epsom salts)	150
Iron citrate	50
Potassium iodide	1

Weigh out the smaller quantities first, and mix well.

The above diet can be prepared once every 2 or 3 days, meanwhile be stored in the refrigerator, but it should only be fed thawed. Small gla earthenware dishes are used for feeding, into each of which 2 oz. of the abo diet are pressed, to be fed last thing each evening. The amount eaten sho be noted each morning, and the dish should be cleaned, to be filled with fr diet the following evening. Another dish containing fresh clean water sho be placed in the cage daily.

This dietary can be relied upon as one on which hedgehogs will thrive w

l it has been improved so as to obviate the onset of certain disorders such as kets, to which, in the course of time, stocks were otherwise found to be prone. wartime conditions, however, it was found necessary to simplify it in a few pects. For large stocks, preparation was found to be not unduly laborious. had one outstanding merit in that it was a clean diet, which obviated soiling the cages such as is likely to occur with simpler foods, such as milk and raw at. The incorporation of raw liver in it, besides adding to its food value, s especially tempting to the appetite of the more fickle feeders. The chalk redient, again, caused the outbreaks of severe rickets to vanish. Some aper form of slaughterhouse offal than is indicated in the diet sheet, such as shed ox intestines, could well be substituted. It is most important to see t that the food, however it is made up, shall not become sour or tainted, and t the cages shall be kept clean by thorough cleaning twice a week. A little sh water should be placed within reach daily, although usually hedgehogs nk very little water. When hedgehogs arrive at the laboratory they are en infected heavily with fleas and other ectoparasites. There is no need to pt special treatment for them, and when the hedgehogs gain condition in tivity they are well able to deal with the parasites by devouring them. As gehogs are nocturnal in habits, they should be given their daily meal in evening, because during the daytime they may after arrival be too sluggish partake of their food until it has slightly turned. Later on, however, they be found to eat ravenously even during the daytime. (This dietary could btless be improved in certain respects. In particular, it has often been nd that some hedgehogs are addicted to gnawing the wooden parts of their es, doubtless as a result of an instinctive urge to maintain their masticatory aratus in healthy order. In view of work since recorded it might be well, bviate this trouble, to feed the breast-of-mutton component of the dietary and separately to each hedgehog. On the above dietary, however, hedge- s have been kept in good health for up to five years, the longest period ed.)

The feeding methods adopted at Oxford are summarized by Ranson (1941) llows :

' Bread, milk and cooked cat's meat are given, prepared as follows. The t is cut into strips, boiled for at least half an hour and minced through a se mincer. 50 g. of this meat and 50 g. of minced bread mixed with ml. of milk is sufficient for a day's food for one pair ; about the same unt is given to a pregnant female or one with young. Mincing is an ortant factor in the preparation of the food. A marked improvement was ced in the condition of the animals after mincing replaced the previous nod of cutting the food into cubes with a knife. The meat should be as as possible, as these animals dislike fat. They much prefer cooked to raw t. Stale bread is used, most of which is household scrap. Boards are ed round the edge of the breeding-cages inside and outside the wire to act ug and worm traps. A rank growth of vegetation is encouraged for shelter to attract insect and small animal life. Occasionally an animal will refuse ed for no apparent reason and even starve itself to death. No explanation be offered for this peculiarity, which occurs more frequently in laboratory- d animals than in those kept in pens outside, and no cure has been found. er records very erratic fluctuations in the growth curves of some individuals."

4. Breeding

Ranson (1941) stated that he had traced only two previous records hedgehogs having been bred in captivity (Herter, 1932, 1933, 1938; Leutsche 1938). He quoted the work of Deansley (1934) who studied the reproducti cycle of the female hedgehog and recorded a low fertility in wild specimen finding that several œstrous cycles might be passed before pregnancy w achieved, but that, when once this had been achieved, few were lost throu failure of implantation.

Ranson's experiences are summarized by him as follows: "It is not y known whether it is the addition of natural food to the diet or whether it is t population-density, increased exercise, or light and temperature extremes whi cause hedgehogs to breed in out-door pens but not in the laboratory. Wh a breeding stock born in captivity has been built up, experiments, which ha been planned, will be carried out to ascertain the factors influencing breeding.

"Many authors have guessed the gestation period to be from 4 to 7 weel In three litters described by Herter the gestation period was calculated to 34 to 49, 34 to 46 and 35 to 42 days. Leutscher records an interval of abo 30 days between observed copulation and the birth of the young, but was n certain of the exact date on which the young were born and, moreover, t parents had been mated before the observed copulation took place. I have n yet ascertained the minimum time, but one litter was born on 16 Augu 1939, to a female (No. 12*) which was mated on 7 July, so that the gestati period cannot be more than 40 days. Another female (No. 4) was mated 28 May and five young were born on 4 July, 37 days later, but as this female h been with another male for some time before 28 May she may have be pregnant before this date. Probably the minimum is very close to Herte suggestion of 35 days.

"While maintaining a breeding colony of voles for ten years, the auth developed a certain skill in counting and estimating the age of embryos palpating the female. In two cases where the embryos of pregnant hedgeh were palpated the age was estimated from their size by assuming that the ra of development of the embryos and the gestation period (21 days) were the sa as those of the vole. This figure was then increased in proportion to Herte minimum figure of 35 days by multiplying it by 35/21. In one case the res was that the young were born on the forecast day and in the other they w born a day earlier.

"If pregnancy is diagnosed by palpation the bladder must not be confu with an embryo. The bladder is nearly always full during the day and fe very much like an embryo in the later stages of development. However, wh several definitely non-pregnant females have been palpated it will be found t it is easy to move the bladder to one side with the thumb of the left hand a feel the uterus and kidneys lying below.

"When the hedgehogs are first obtained they should be placed in the o door breeding-pens as soon as the sexes have been determined. If necess they can be cleared of ectoparasites by anæsthetizing them with anæsthe ether and shaking them over a large sheet of paper. The ticks and fleas m be burnt or drowned, as they revive after ether almost as quickly as the hed hogs. If cage space is not available for arranging the hedgehogs in pa harems may be set up, but there is a slight risk of loss from fighting or bully

vo or more males should not be put in the same cage with females, but males ll live together if there are no females near.

" Mating can be expected in April and May, and the first litter should be rn in May or June. The second mating occurs in July or August, and the cond litter should be born in August or September. No post-partum œstrous cle occurs in the hedgehog (Deansley, 1934), so that there is no advantage in ving the male with the female after she becomes pregnant ; in fact, there is definite disadvantage in leaving the pair together, as the male may destroy e young or so disturb the female that she neglects them. The pregnant nales can either be put into the small outdoor movable cages or into bins in e animal house. When the young are expected the nest can be examined ring the evening when the female is out feeding ; but this must be done very efully and the nest must be covered over again when the count is complete. the female is removed from the nest during the day and the young are counted e will kill or desert them. This has happened on two occasions when the sts of females 1 and 16 were unintentionally disturbed during the daytime. th litters, which were alive and well, were killed by the parent.

" Herter records that the last time he observed a female give milk was en the young were 42 days old ; and he observed one young of 21 days old ing solid food. Litters have been weaned at from 38 to 44 days old with nplete success. There is some evidence that 40 days is the best time, as er this the female is not giving food but is competing unfairly with the ng for the food provided. The young have been examined during the day- ne without the female objecting when they were 5 days old, but it is probably er to wait until they are at least a week old before they are examined during vlight hours. All the twenty young which were not killed at birth were aned, the sex ratio being thirteen males to seven females. In four litters of *europæus* bred by Herter twenty young were produced, twelve males and nt females. It therefore appears as though the sex ratio were biased in our of males. Herter has described minutely the growth and development he young and illustrated it with some charming pictures, so it is not neces- y to redescribe this part of the cycle. All that need be said here is that no tter how fast the young grow they are incapable of breeding in the season which they are born (unless they can eventually be made to breed in the ter)."

5. Hibernation

In hedgehogs kept at a constant temperature (65° F.) and fed as above the et of hibernation can be prevented during the normal hibernating season nter). However, experience has shown that the normal seasonal rhythm nly superficially masked in this way, and that the response to certain ctions is not identical with that which occurs in hedgehogs that are normally ve.

Ranson (1941) recorded his observations on this subject as follows : " It es no difference to the hedgehogs whether they hibernate or whether they nd the winter in a warm animal house in a fully active condition. For nomy of food it is probably better to let them hibernate, for as yet no litters e been produced in the winter.

" The young should be brought into the laboratory as soon as the cold ther starts, and kept there at least until the New Year, i.e. until they have

grown to adult size of 500 or 600 g. Then they should be put outside for a sho hibernation. Without this additional growing-time it is possible that tl young would take two years to reach sexual maturity, for those left outsi until January are very puny little things of about 350 g. If, on the other han they are kept in the laboratory all the winter in a fully active condition, th may begin to lose weight after January. This may be due to a diet deficien or to an unnaturally lengthy growing-period.

" During very cold weather no food need be put into the pens containi hibernating hedgehogs, but during mild weather it is important to keep a sm food supply always available as they will sometimes break hibernation and e ravenously. The food need not be wasted if uneaten as it can be used f hedgehogs in the laboratory and a fresh supply can be put outside."

For facts concerning the physiology of hibernation, see Suomaleinen (193 1938 *a*, *b*, *c*, *d* ; 1939) ; Herter (1938).

It was our experience at Pirbright that it is not often easy to cause t onset of hibernation in all hedgehogs at will, or to maintain it unbroken throug out the winter, especially during mild spells. The practice adopted was transfer each hedgehog to a dark cold chamber, at a constant low temperatu in a separate bin, with abundant hay. It prepared itself for hibernation enveloping itself tightly, onion-wise, with layers of hay. Some food was ke near it so as to obtain ready evidence of any break in hibernation from t disappearance of the food.

6. Handling

Ranson, who was experienced in handling hedgehogs, recorded his obser tions on this subject as follows (1941) : " When freshly caught, hedgehogs a the most disconcerting of all laboratory animals. Their wall of spines cann be breached even by patience or guile. If they are anæsthetized to remo ectoparasites, opportunity can be taken to make a thorough examination them. After a few weeks in captivity, if they are handled gently when th come out to feed, they will become fairly tame and remain uncurled wh picked up. The females are always more obstinate than the males, and it tak much longer to win their confidence. The technique for handling adu hedgehogs is as follows : The animal is placed on a flat surface and the spin over the rump are tickled with the left hand. This makes the animal lift its head, and the scruff of its neck can be gently grasped with the right hand placing the first finger and thumb in the ears. It can now be gently lifted so that it is standing on its hind legs. If it starts to curl up again the back a the rump should be tickled once more without releasing the grip with t right hand.

" When the hedgehog is standing up slide the left hand gently under t belly and the animal can be lifted up, held by the neck with the right hand a resting on the left hand. Sexing or palpation of embryos is now easy, as t animal will almost certainly remain extended.

" If a large number of hedgehogs are being dealt with and there is no ti to study the idiosyncrasies of individuals, then force will have to take the pla of guile. For this an apparatus, like a trousers-press made of two wood frames covered with wire netting is used. These frames are hinged at one si in such a way as to leave a space of about 2 in. between the upper and t lower frame when the apparatus is completely closed ; the frames are h

gether when closed by an adjustable catch. The press is opened wide and e hedgehog is encouraged to sit on one of the frames and uncurl as far as it ll by tickling. The other frame is then pulled over and pressed down firmly to the back of the animal and the catch secured. The hedgehog is now in a ge compressorium, and by turning the press over the underside is accessible rough the netting for examination, inoculation, or for taking vaginal smears. the press is used gently but firmly the hedgehog cannot hurt itself nor can it se up, as its claws and spines anchor it quite effectively. Of course, it is possible to palpate embryos in a hedgehog which is in a press, but if a mated nale is very obstinate it can either be weighed regularly together with its te, a sudden increase in the weight of the female being used as an indication pregnancy, or it can be anæsthetized periodically. This latter method is, wever, not recommended owing to the uncertainty of the part played by the turbance factor in preventing animals from breeding."

At Pirbright the method of causing a hedgehog to uncurl is as follows. e back bristles near the neck are prodded very gently with the point of a pair strong artery forceps until the hedgehog begins to uncurl, the fingers of the hand are then gently insinuated between and around the hocks, which are v drawn back, and the artery forceps are next fixed on to the bristles behind head ; the hedgehog is then lifted up and pulled out straight.

[Hedgehogs may suddenly utter a peculiar cry like the scream of a child, ich may be very disconcerting to the worker who is not prepared for it. ckhart (1944).]

7. Anæsthesia and Euthanasia

At Pirbright the most successful method was found to be the intravenous ection of chloral hydrate into the external saphena vein just as it curls nd above and outside the hock. With a fine hypodermic needle, the hedge- being held steadily, this injection can be made without difficulty. The e, of a 5-per-cent. or 10-per-cent. solution, was varied according to the depth anæsthesia required, an overdose being given to produce euthanasia. hanasia was also produced by intraperitoneal injection of 5 or 10 ml. of the tion prior to the excision of various tissues required for pathological estigation. [In destroying young hedgehogs painlessly by the intraperi- eal injection of nembutal, there is a period of deep narcosis preceded by a nite loss of the curling and other reflexes ; this suggests that nembutal ld be an excellent anæsthetic.—*Ed.*]

8. Diseases

Experimentally the hedgehog is employed for the study of foot-and-mouth ase (Hulse & Edwards, 1937 ; Edwards, 1937 ; Wolf, 1939) and of yellow r. Edwards (1936) was able to set up a catarrhal condition by intranasal llation of nasal discharge from human patients with an acute coryza and as soon afterwards found by Stuart-Harris that the hedgehog can be readily cted with the virus of human influenza. Certain of its natural infections to spread rapidly in captivity unless careful management is practised.

Respiratory disease due to infection by *Brucella bronchiseptica*. This is a mon and distressing condition, and it is especially evident among stocks ed together at the approach of winter. It was for this reason mainly from the moment of captivity the hedgehogs at Pirbright were kept in

separate compartments designed as above to diminish the risk of spread. O examination of normal stocks, a large proportion are found to be carriers *Brucella bronchiseptica* in the respiratory tract, the incidence and intensity invasion tending to diminish during the warmer weather. The disease set u when, through improper management, the infection was allowed to obtain serious foothold among stocks was a catarrhal or broncho-pneumonia, ofte with profuse nasal discharge. In fatal cases, secondary bacterial invader such as Pasteurella and hæmolytic streptococci could be readily isolated fro the upper respiratory tract.

Enteritis due to *Salmonella enteritidis, Gaertner.* Hedgehogs are again ver commonly carriers of this organism and the infection is often lighted up durin a virus attack, *S. enteritidis Gaertner* being then readily isolated in pure cultu from the mesenteric glands and the blood. The strains isolated at Pirbrigh showed a marked tendency at first to grow firmly adherent to the cultu medium when surface-cultured on solid media.

Parasitic infestations. Lockhart (1944) records the death of a hedgeho from what appeared to be " gapes." The trachea and bronchi were filled wit gape-like worms which were presumably *Crenosoma striatum.* Taylor (194 states that *Crenosoma vulpis* is known to be pathogenic to the silver fox an that probably *C. striatum* also would be dangerous in large numbers; sinc captive hedgehogs have little contact with land molluscs, the probable inte mediate host, he considers that this condition should not cause much troub to experimentalists. Ticks (*Ixodes*) are common on newly caught wild hedg hogs (see Chapter 3).

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CHAPTER 18

THE PIGEON

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1. Supply of pigeons. 2. Accommodation. 3. Nutrition. 4. Breeding. 5. Diseases.

1. Supply of Pigeons

Although pigeons are not used generally as experimental animals they are ed for certain purposes such as the biological assay of prolactin, investiga- ns in connection with the vitamin-B complex, and the production of pigeon- x vaccine for poultry. It is usual for laboratories to obtain their require- nts from commercial pigeon-breeders rather than to breed their own birds. less the laboratory concerned uses pigeons frequently and in large numbers elf-contained breeding unit is hardly an economical proposition.

There are numerous varieties of pigeon, many of which have been developed exhibition and have exotic characters which render them unsuitable for oratory work. The breeds used for squab-production for the table are the st suitable for investigational purposes. Breeds of this type are Kings, neaux, Swiss Mondaine, and Hungarian. The adult males of these breeds gh between 24 and 26 oz., and the females weigh about 22 or 24 oz. Their abs should be fully feathered and independent of their parents at four weeks age. The Homer pigeon, although a racing bird, is also useful. It is not isable to use cross-bred pigeons, especially in biological assays and nutri- nal work for which a standard bird is required.

2. Accommodation

Accommodation and all other aspects of the husbandry of the pigeon are austively discussed by Levi (1941) and more shortly by Lee (1930). There many varieties of pigeon loft, but whatever accommodation is used it must well ventilated, dry and sheltered from the prevailing winds, and when sible the flight should face south.

The pigeon lofts which are described in this article consist of fourteen pens, en on each side of a central gangway. Ten of the pens are in constant use breeding units and the remaining four are used to keep the young birds re selection as future breeding-stock or as issues for experimental work. re are also two small rooms, 9 ft. 3 in. × 5 ft., which are used as food and w stores. The clean sand is kept under cover in an outside shed. Each houses from 20 to 25 breeding pairs.

One attendant working full time on these lofts is able to perform all the ine cleaning and feeding duties as well as to keep the necessary records. attendant should be a quiet and patient worker as the birds are difficult

FIG. 49.—PIGEON LOFTS AT COMPTON.

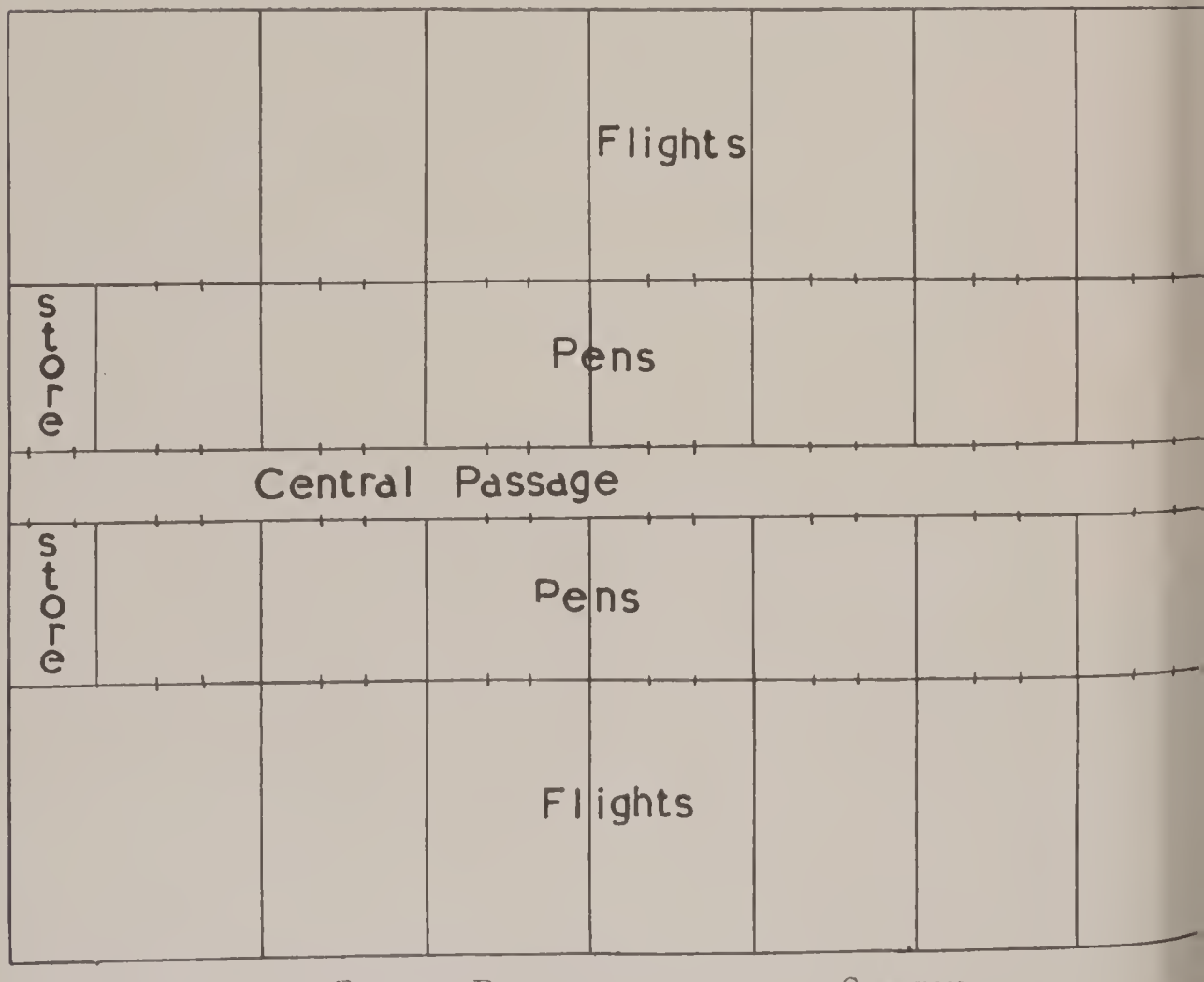

FIG. 50.—PLAN OF PIGEON LOFTS AT COMPTON.

catch and they are easily upset. A large-sized catching-net with a mouth out 18 in. in diameter should be used to catch the birds.

These pigeon lofts are built of brick with a corrugated asbestos roof. The ns are identical in size measuring 9 ft. 6 in. wide × 9 ft. 3 in. deep with a ding door 2 ft. wide opening on to the central gangway. A sliding door in e opposite wall opens into the wire flight. The height of each pen is 12 ft. ping to 8 ft. 6 in. at the eaves. Under the eaves there are six pop-holes ening into the flight, with alighting platforms inside the pen and outside in e flight. The inner platform is hinged so that when necessary the pigeons n be shut in the pen. Each pop-hole measures 9 in. × 10 in.

A pen has two sets of nesting-shelves, each set comprising sixteen nests

DIAGRAMMATIC VIEW OF POPHOLES.

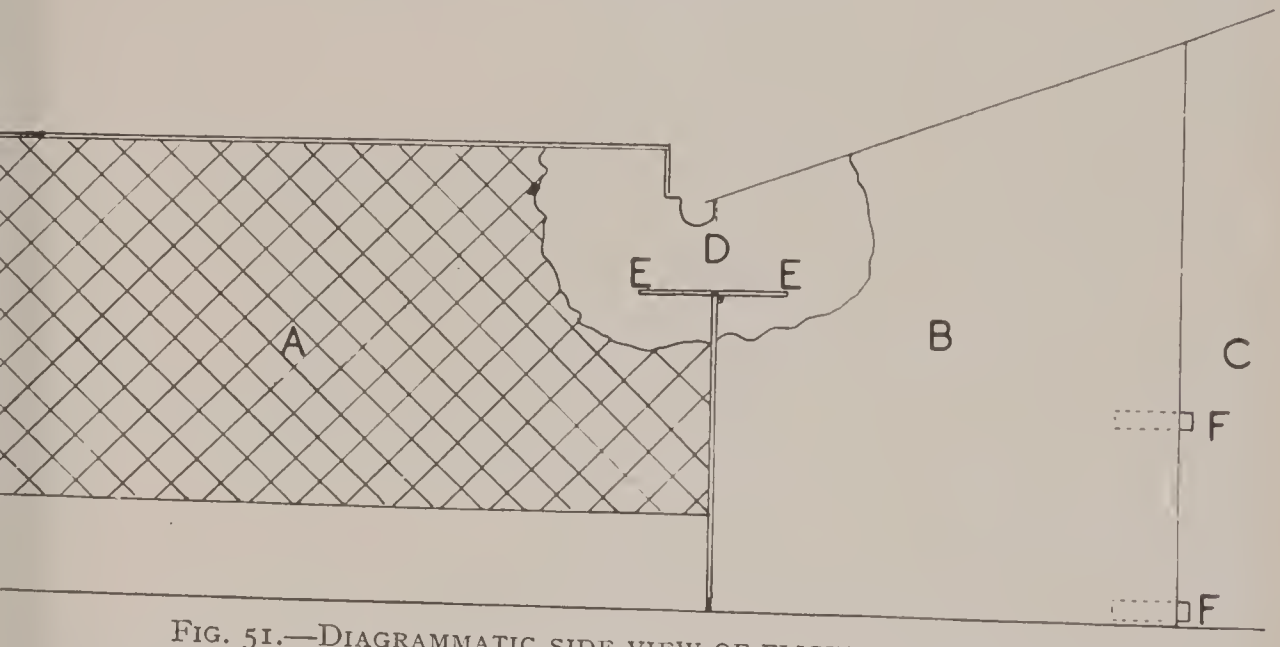

FIG. 51.—DIAGRAMMATIC SIDE-VIEW OF FLIGHT AND PEN.

A—Wire flight. B—Pen. C—Passage. D—Pophole. E—Alighting board. F—Water trough.

ced against the partition wall. The fittings are made of wood and are ovable. Each nest consists of a sliding shelf $23\frac{1}{4}$ in. long × $15\frac{1}{4}$ in. wide, which is a T-shaped piece of wood which divides the shelf into two halves. s T-piece allows the parent birds to select either half of the shelf for their and helps to keep the nest together. Some pigeon-breeders use porcelain ls for this purpose. The passage wall has two apertures through which are ed drinking-troughs, 18 in. long × $4\frac{3}{4}$ in. wide × 4 in. deep; one is at r-level and the other at a height of 4 ft. Thus the watering can all be done n the central gangway. The sliding doors opening into this gangway are d in their top halves so that the attendant may be able to observe the ons without entering the pens. The outer doors leading into the flights e wired glass windows let into them.

The feeding-table in each pen consists of a shallow tray surrounded by

alighting-boards raised about 20 in. above the ground. Underneath the tra and boards are racks in which is placed straw for the pigeons to use for buildin their nests.

The flights have alighting-boards along their wire sides. The ground o the flights is gravelled, allowing the droppings to be washed through to th earth beneath.

Pigeons endeavour to keep themselves clean, and bathing facilities mus

FIG. 52.—PERSPECTIVE DRAWING OF NESTING-SHELVES.

be supplied in each flight. The bath provided is made of galvanized met measuring 30 in. × 18 in. × 4 in. The bath is raised above the ground abo 3 ft. and is provided with a central plug for drainage. A tap is provided imm diately above the bath, which two or three times a week is filled in the mornir and emptied in the early afternoon. In cold or windy weather the pigeo will not bath. If bathing accommodation is not provided the birds will bath in their drinking-water troughs.

Cleaning. Clean lofts are an essential part in the successful management of geons. The feeding-tables and nest shelves should be scraped daily, and the cumulated droppings on the sanded floors should be raked out. The nests which there are young squabs must be kept as clean as possible without sturbing the young birds. It may be necessary during the period of rearing renew the nesting-material if the nests become very wet, but the squabs ould be handled as little as possible. The sand on the floor is replaced proximately once a month. The fresh sand should be dry when it is spread the floor. The soiled sand may be used as manure on the land. The gravel the flights should be raked four or five times a year to prevent foulness from cumulated droppings and to facilitate filtration through to the earth beneath. e gravel must be changed every two years. The pens are thoroughly

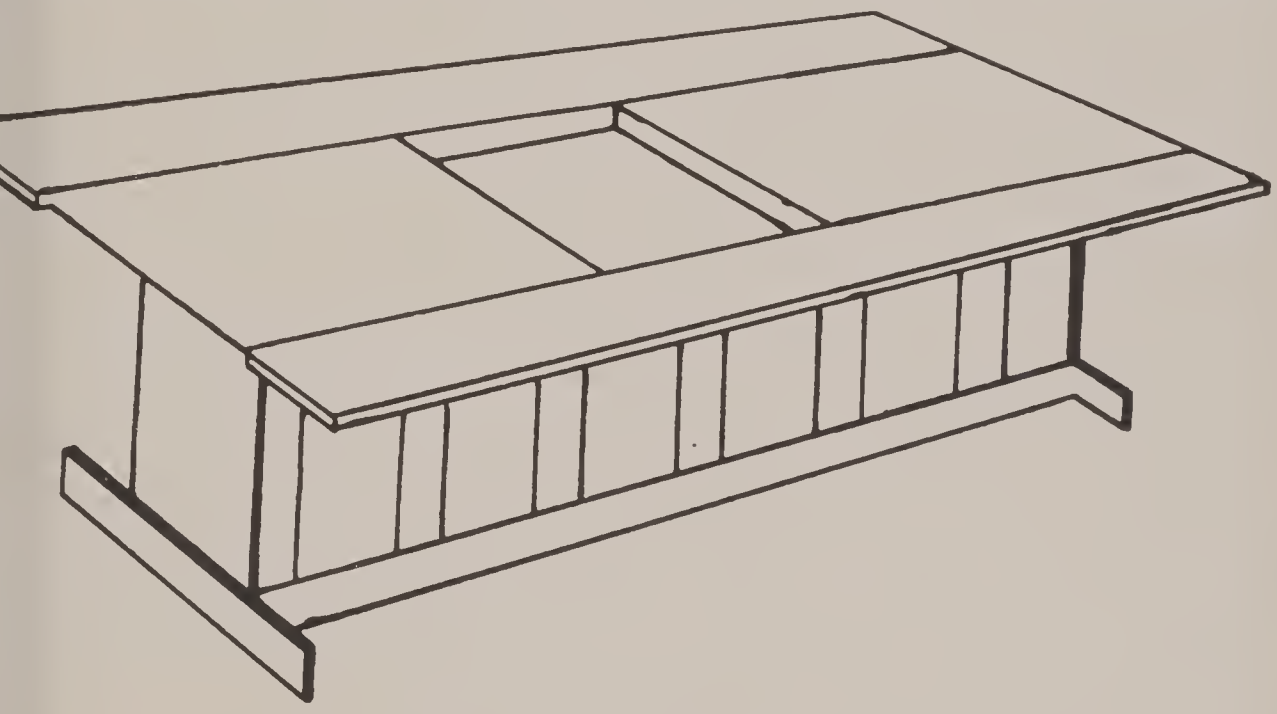

FIG. 53.—FEEDING-TABLE WITH STRAW RACKS.

ned once a year. The walls are scrubbed, sprayed with a 5-per-cent. tion of formaldehyde, and whitewashed. The wooden fittings are scrubbed creosoted.

3. Nutrition

The pigeon has been extensively used in nutritional investigations and there onsiderable literature on its nutritional requirements. However, many of e experiments were of a very artificial type and it is difficult to translate findings into terms of practical pigeon-feeding.

The work of Carter & O'Brien (1939) and others has established that the on requires thiamin (vitamin B_1), riboflavin (vitamin B_2), pantothenic (vitamin B_3) and pyridoxine (vitamin B_6). Dam, Schonheyder & is (1937) have shown that the anti-hæmorrhagic vitamin K is necessary he pigeon. Ascorbic acid (vitamin C) is synthesized by the pigeon and r poultry and it is not an essential constituent of the diet. Vitamins A D are both required by the pigeon.

However, for the purposes of practical pigeon-breeding the following diet recommended by Lee (1930) have proved satisfactory :

(A)	Small whole maize	4 parts.	(B)	Small whole maize	4 parts.
	Kafir	3 parts.		Egyptian maize or milo	3 parts.
	Field or Canada peas	3 parts.		Maple peas, peanut kernels or garden peas	3 parts.
	Hard red wheat	1 part.		Hard red wheat	1 part.
	Millet or hempseed	1 part.		Hempseed or flaxseed	1 part.

Small quantities of rice, rape, canary, vetch and chopped sunflower seeds ma also be fed for variety. The grains should all be of a good hard quality A combination of grains, besides being necessary for a fully balanced die enables the adults to pick out the most suitable grain for the size of squab the are rearing. The birds are fed twice daily in the feeding-table trough and a given as much food as they will clear up in twenty minutes. These amoun work out to approximately 1 lb. per pigeon per week. When possible it desirable to hand-feed the birds in order that they may become used to peop and consequently easier to handle.

During the war, the diet had to be restricted to the National Pigeon Foo which consists mainly of peas and maize in the proportion of 75 parts to parts. Occasionally barley and oats are added in small proportions. winter a good-quality cod-liver oil soaked on wheat should be supplied once week. Each bird is allowed $\frac{1}{4}$ oz. of wheat soaked with 1-per-cent. cod-liv oil. Pigeons require grit, oyster shell and common salt, and they appreciat a small quantity of charcoal ; these ingredients are supplied as proprieta mixtures. The manufacturers' instructions for feeding should be adhered t as too much salt may prove harmful. We have found that birds which a used to one particular mixture of grain are difficult to feed if an alteration made, and they will go hungry to begin with rather than feed off an unfamili food. Changes in diet should be effected gradually.

4. Breeding

Pigeons breed most of the year, except when they are moulting during th months of June to August. Therefore it is unnecessary, except in extrem climates, to provide artificial heat in the pigeon lofts. The pigeon is monog mous and consequently each breeding-pen must contain equal numbers males and females. It requires some experience to differentiate the sexes a this can best by done by watching their behaviour. The male struts abou cooing and dragging his tail, the female seldom coos and holds her body mo horizontally than the male. The male is larger and coarser, especially in t region of the head and neck. It is also necessary to sex the squabs if o wishes to avoid having pens containing young pigeons of mixed sex. It possible to do this when the squabs are three weeks old and still in the nes At this stage the male will have a wider space between the eyes than the fem and the top of the head is flatter. The squabs having been sexed, the se should be distinguished by differently-coloured leg bands so that at weani the males may be penned separately from the females until they are old enou for mating.

Pigeons may be mated for the first time at between 7 and 9 months. Birds y be paired by confining a male and female in a nest with a wire front with ough food and water for two days. This is usually long enough for the ds to mate and when they are released they should begin to build a nest with aw. As the birds are mated they have numbered identification rings put their legs. When all the pairs in the pen have mated any surplus nests uld be removed or shut off. Normally matings will remain the same, but here is some disturbance the cock may choose another mate. If one bird a pair dies its mate must be removed or remated immediately. On no ount should an unattached male be left in a breeding pen.

Female pigeons usually lay two eggs : one egg is laid one day and the nd about 48 hours later. The incubation period is 17 days. Both birds on the eggs, the male during the morning and early afternoon and the ale for the rest of the 24 hours. At the fifteenth or sixteenth day of ubation the parent birds begin to secrete pigeon milk from the cells lining ir crops. For the first four days after hatching the squabs are fed by the ents on this secretion. Then they are fed small grain by the parents. wth is extremely rapid and at 10 days of age they are eating whole grain. 3 or 4 weeks the squabs should be weaned, sexed and placed in the appro- te pen in a straw nest on the floor with a small food-container near them.

If the breeding performance is up to the average the breeds mentioned ve should produce 7 or 8 squabs a year, and some pairs may produce r 11 squabs. Enough young birds should be retained each year to replace ut 20 per cent. of the breeding stock. Pigeons are most prolific between ages 2 and 5 years, although many will breed up to the age of 8 years.

5. Diseases

The pigeon is naturally susceptible to a number of infectious diseases ch may be broadly grouped as follows : (1) bacterial infection (salmonello- ; (2) virus infections (pigeon pox and psittacosis or ornithosis) ; (3) pro- an infections (coccidiosis and trichomoniasis) ; (4) helminthiasis (chiefly riasis and capillariasis of the digestive tract) ; and (5) external parasitic ctions. The relative importance of these diseases is hard to define in the nce of extensive published records of routine post-mortem examinations. vever, Lesbouyries (1935), in a large number of post-mortem examinations rrier pigeons, found 2,936 cases of coccidiosis and round-worm infestations, 4 cases of pigeon pox and (?) diphtheria, and 1,349 cases of paratyphoid se. Hare (1937) analysed his findings in 110 consecutive cases of disease igeons. The most frequent infections were helminthiasis (especially laria) 19 cases, coccidiosis 17 cases, and pigeon pox 12 cases. Among the ining 23 diseased conditions that were diagnosed no single condition reached le figures.

almonellosis (paratyphoid). Salmonella infection of pigeons is common, these organisms may frequently be isolated from pigeons which have died some other condition such as psittacosis, pigeon pox or trichomoniasis. e is a considerable literature on the subject of pigeon paratyphoid, some nich is quoted by Fenstermacher (1944) and Orr (1932).

n squabs the disease usually occurs at about 2 weeks of age and death follow within 48 hours of the beginning of illness. In older birds symptoms

may be prolonged and varied. There may be shaking of the head a twisting of the neck. Breathing is often laboured. Discoloration of the ey is not uncommon, and diarrhœa is a frequent symptom. Recovered bir may become carriers of the organism, and such birds may suffer from arthri of the leg and wing joints.

On post-mortem examination the liver and kidneys will be found to swollen and congested. The liver is usually yellowish in colour and contai a number of small necrotic foci. Pneumonia is a common lesion, the lun being brown in colour. The spleen may be enlarged, although this is not constant feature. Enteritis is often present. The pathology of the disea has been described by Cash & Doan (1931). In the acute form of the disea the causal organisms can be isolated from the blood, liver and bone marro They may also be recovered from the synovial fluid of affected joints. It well established that the organisms may be transmitted via the ovary and th may be recovered from the egg. Salmonella may also be isolated from t pharyngeal fluid of infected adults and from their fæces. Thus there are number of possible methods of transmission of the disease to the you unaffected pigeons.

It should be pointed out that the use of infected pigeon eggs for cooki has caused food poisoning in man (Clarenburg & Dornick, 1932).

The Salmonella type responsible for this disease in pigeons is a variant *S. typhi-murium*. According to Edwards (1935) this variant lacks the soma antigens V and XI (Kauffmann-White schema) and possesses only the soma antigen IV. Edwards states that this variant in pigeons is universal in dist bution. The type has been designated *S. typhi-murium var. Storrs*.

Control measures depend on the detection and elimination of the ad carrier birds by means of repeated agglutination tests. Strict hygie measures also must be undertaken. According to Lesbouyries & Ver (1932) preventive vaccination has had a varied degree of success.

Virus infections

Psittacosis (Ornithosis). Coles (1940) in South Africa was the first to dr attention to the existence of psittacosis in pigeons. In the same year Pinkert & Swank confirmed its existence in the U.S.A. The first description of disease in these birds in this country was by Andrewes & Mills (1943). Fr these and other references in the literature it appears that the virus is fai widely distributed in pigeon flocks.

The disease has no clinically distinctive features. It is primarily an inf tion of immature pigeons perpetuated by recovered carrier adults. Dea frequently occurs without premonitory symptoms. In some lofts the dise may be responsible for 50 per cent. of the young birds failing to reach matur (Hughes, 1946).

In the young bird the morbid process is principally an inflammation serous membranes. Pericarditis and peritonitis are the commonest lesio Suitably stained smears of the exudate will be found to be rich in virus elem tary bodies. The liver may be enlarged and bronzed with numerous necr foci. In older birds the spleen is often enlarged, measuring from 1 to 5 cm length. A full description of the disease in birds, including the pigeon, is gi by Meyer (1944). Infection with the virus may be accompanied by infect with *S. typhi-murium var. Storrs*.

Our knowledge of the disease is still fragmentary, and methods of controlling e infection must be largely empirical. Attempts to detect latently infected rds by means of the complement-fixation test might be made, but not all fected pigeons' sera contain antibodies (Meyer, 1944). However, the com- ement-fixation test is of value in confirming the presence of the disease in e loft. Although the pigeon virus appears to be less virulent for man than e classical psittacine type, infection of man from pigeons has occurred nadel, Wall & Gregg, 1943). Any influenza-like infection in the pigeon tendants or other persons in contact with pigeons should be regarded with spicion until ruled out by at least two negative complement-fixation tests. rise in complement-fixing titre during the course of the illness helps to nfirm the diagnosis.

Pigeon pox. This disease is fairly common in pigeon lofts, and usually ects birds at about 6 or 7 months of age. It is not often seen in squabs in nest. The causal agent is a filterable virus which, although similar in some pects to other avian pox viruses, is a distinct entity as it produces the natural ease in pigeons only.

Wart-like excrescences appear on the legs and feet. Lesions of a cankerous pe may be seen at the commissures of the mouth and these lesions may end to the trachea with fatal results (Brunett, 1944). However, Lesbouyries 35), states unequivocally that the diphtheroid lesions which occur in the pphagus of the pigeon are not caused by pigeon pox virus but are due to homonads. It appears that the membranous type of the disease as seen in l pox is not often observed in the pigeon.

Diagnosis may be made from the appearance of the typical lesions, but if essary this may be confirmed by the inoculation of ground-up material n the lesions into the feather follicles of another pigeon. In positive cases usual epitheliomatous lesion develops at the site of inoculation.

Prevention of the disease is satisfactorily accomplished by the use of eon-pox vaccine. Squabs should be vaccinated at about 6 weeks of age. vaccine is applied to the breast region by painting it over five or six eathered follicles with a brush.

TOZOAN INFECTIONS

Trichomoniasis. (Canker or yellow button.) Infection with trichomonads serious problem in some pigeon flocks. The disease usually affects squabs, ough cases may occur in adults. The predominating lesion is a fibrinous ntheritic inflammation of the anterior part of the digestive tract. The ns are commonly limited to the pharynx, œsophagus and crop. Caseous cesses may also be found in the liver, pancreas and lungs. In some birds e may be an extension of the process to the accessory cavities of the head the air sacs.

Schaaf (1939) states that infection in the squab may arise through the navel, ng rise to the lesions of the abdominal viscera. However, infection usually rs by ingestion. The sources of infection are clinically healthy adults may harbour trichomonads. The squabs may be infected directly by parents during the process of feeding them with crop milk, and drinking- er is another vehicle for transmitting the parasites.

The trichomonad which gives rise to these lesions is commonly called *olumbæ*, but according to Stabler (1938) the trichomonads of the pigeon

and the turkey are identical and the correct name is *T. gallinæ* (Rivolta, 1878). This protozoan has four free anterior flagellæ and a somewhat elongated body. In fresh wet preparations made directly from the live or recently dead bird the trichomonads will be actively motile. Active trichomonads cannot easily be demonstrated if the bird has been dead for some hours.

Clinically affected birds should be eliminated or treated. Healthy carriers also should be treated. Squabs hatched by infected adults should be given to healthy adults for rearing. Miessner & Hansen (1936) recommended 0·5-per-cent. " sulpholiquid " and 0·5-per-cent. copper sulphate for the disinfection of the drinking-water. For treatment Kraneveld & Nasoetion (1940) obtained good results with doses of 0·5 ml. of 10-per-cent. sulphanilamide administered intramuscularly or directly into the local lesions. The same dose was repeated three times on alternate days. The application of sulphanilamide paste to the local lesions was equally effective. Bingham (1944) has reviewed the literature on trichomoniasis, and her article contains numerous references to the disease in pigeons.

Coccidiosis. This disease appears most frequently in young pigeons under six months of age, causing emaciation, anæmia and a green, watery and slimy diarrhœa. It is usually limited to the duodenum. (The cæca in pigeons are rudimentary.) The essential lesion is a catarrhal inflammation of the mucosa of the duodenum, in which the coccidial oocysts are numerous. Hall (1937) has described lesions in the liver in pigeons in which he demonstrated coccidia.

Good sanitation and the best dietary conditions possible are indispensable in a control programme. Sulphamethazine in the drinking-water has been found successful in the treatment of the disease in chickens by Horton-Smith & Taylor (1943). These workers recommend a saturated solution of the drug in place of drinking-water, but with young pigeons it might be necessary to reduce the concentration.

Helminthiasis

Infestation of the intestines with the hair-like worm *Capillaria columbæ* (*Trichosoma columbæ*) produces all the usual symptoms of intestinal parasitism. The birds are lethargic, tending to huddle on the ground, the vent feathers are ruffled and soiled, and there is paleness of the skin and visible mucous membranes. These symptoms are usually accompanied by emaciation and diarrhœa. The watery fæces contain flecks of mucus and in some cases blood.

In advanced fatal cases the intestinal mucosa shows considerable destruction with sloughing of the mucous membrane.

Hygienic measures to reduce re-infestation to the minimum must be adopted.

Infestation of the small intestine with ascarid worms also occurs frequently in pigeons. Droopiness, emaciation and diarrhœa are the usual symptoms. On *post-mortem* examination the intestines are found to be dilated and thickened. Occasionally the infestation may be so gross as to cause a rupture of the intestine followed by a fatal peritonitis. Lesbouyries (1935) recommends the following treatment for both types of infestation : equal parts of areca nut and kamala in pill form given in doses of 20 to 30 cg. on three consecutive days and repeated in fifteen days.

XTERNAL PARASITES

Lice (*Columbicola columbæ, Goniocotes compar, Goniodes damnicornis*). The ntrol of lice in pigeons must be on lines applicable generally for the control these parasites in birds. The extent of the infestation depends to a con-lerable degree on the general health of the birds, and every effort must be ade to remedy any unthriftiness as well as to direct attacks on the lice. secticides may be applied to the pigeons either by a wet dipping method or dry dusting. Sodium fluoride or sodium fluosilicate are the best agents for is purpose. We are not aware of any work in which D.D.T. has been tested pigeons, but this substance should be effective. Owing to the fondness of geons for bathing, sodium fluoride may be incorporated in their bathing ter. To make certain that all birds immerse themselves, opportunities for thing should be withheld for 2 or 3 days prior to the treatment. The agents ould be mixed in the water in the proportion of 1 oz. per gallon of water. sting methods also are satisfactory, although they are more laborious. The wder is applied to the head, neck, back, breast, vent and tail, and under each ng and leg, and is rubbed into the feathers with a ruffling motion. The plications should be repeated at 10-day intervals until the infestations have en controlled.

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Note added in proof. W. F. Hollander (1945) has recorded " A type of psychopathy
the domestic pigeon " (*J. comp. Psychol.* 38, 287–289). In cages of 48 cu. ft. a
24 cu. ft. volume breeding behaviour is normal, but when a pair is confined to cages
12 cu. ft. or less there is usually abnormal behaviour. Violent fighting often develo
with serious injury or death of the female and with loss of eggs or young from tram
ling. The behaviour seems to be associated with the reproductive cycle and partic
larly with incubation. It has been noted in a number of breeds, and especially
common mongrels, but not in captive ring-doves.—*Ed.*

CHAPTER 19

THE CANARY

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With a section on Diseases

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1. Introduction. 2. Characteristics of canaries. 3. Accommodation. 4. Nutrition. 5. Breeding. 6. Cleaning. 7. Moulting. 8. Colour feeding. 9. Diseases.

1. Introduction

Canaries have not in the past been widely used in research institutions and oratories, although within recent years certain laboratories in this country ve employed them in connection with the examination of therapeutic and ic substances and of diets of pet birds. Usually canaries are kept as gsters, and in certain parts of Europe and the British Isles the different ieties are bred by local breeders on a substantial scale. As often as not the oby—for such it tends to be—is handed on from father to son. Meetings of al breeding societies are regularly held and they organize canary shows at ich not only are prizes awarded, but also many of the birds are purchased to vide breeding stocks. It is in this way that champion breeders often uire their best birds for breeding. In peace-time an annual show was held the Crystal Palace, and the awards of this show were highly prized by ary breeders and fanciers. On the continent of Europe the main breeding ality is in the Harz Mountains, where, as in this country, hundreds of private eders are to be found. The Harz Mountain birds were widely sold in the ited States before the war, mainly because they were cheaper than birds n the British Isles.

In view of the manner in which canary-breeding is carried out, it is inevitable t the process should be governed largely by rule-of-thumb methods, with judices in favour of certain ways of breeding, and above all a general lack of andard procedure. This does not mean that there are no common factors, concerning food, which are agreed upon by many breeders. It does mean, vever, that most breeders, having acquired their knowledge by bitter erience of the losses which can be incurred in breeding canaries, stand by r own methods and can only with difficulty be persuaded to modify them. tain superstitions of the breeder illustrate his conservatism. Thus, it is that it is unlucky to count canaries, as deaths always follow.

It cannot be said that the best methods of managing canaries have yet n formulated. In this section, however, it is hoped to present facts con- ing the birds which may be helpful to any research worker who finds it essary to employ them for scientific purposes.

2. Characteristics of Canaries

For the scientific purposes of the research worker it is perhaps unnecessa to distinguish between the different varieties. The original canary, a native the Canary Islands, was a wild bird quite different in colour from the yell household pet. It was buff-green or yellow-green in colour, unrecognizable many persons as a canary. It was only by intensive breeding that the comm canary-yellow bird was developed. Indeed even to-day a person familiar on with the yellow variety would be astonished at the different colours possil in home-bred canaries. They include, in addition to the well-known yello pure white, blue, cinnamon and buff, as well as variegated types.

Canaries are usually of a nervous disposition and are particularly liable be affected by strange influences. This is especially so during the breedi season (in this country, between March and July). They are so easily frighten that this disposition is a real problem in breeding, and it is one of the importa causes of the so-called " bad luck " which is often encountered. Canaries li a quiet life and dislike approaches by strangers and this fact is of fundamen importance. The sudden switching on of a light when a canary has you may be accompanied by a momentary panic which causes the bird to tread its young and injure or kill them. The precautions to be taken in these a in other circumstances will be referred to in the following paragraphs.

The three best-known varieties in this country are the Norwich, Yorksh and Border. The Norwich bird is large and has a full and bold shape. T Yorkshire, also a big bird, is slimmer and less curved than the Norwich. T Border is definitely smaller but is a hardy variety. Modifications of each s include crested birds and the fancy varieties with a cap of feathers on the he All can be bred out-of-doors, but the best results have so far been produced indoor breeding and management.

3. Accommodation

The essentials of a building intended for the housing of canaries are sim and apply to most caged birds. The room should be light, with good venti tion. The temperature should be controlled, if possible, between about and 60° F. (10° to 15° C.). While canaries can stand a fairly cold room, th dislike draughts and are liable, if exposed to them, to contract respirat complaints which are often fatal. If, on the other hand, the room is too ho bird is liable to go into a soft moult which usually leaves it in a sub-norm state of health.

Cleanliness must be strictly observed in order to prevent infestation w parasites, and the ideal room is one that lends itself to easy cleaning a disinfecting.

Cages. The construction of the cage is of great importance in ensuri healthy stock, and there are several points which demand particular attentio

The average show cage of the person who keeps a single canary for its so capacity is of little interest to the research worker. In such cages, wired round as they are, and made for beauty rather than utility, the bird is not its ideal surroundings owing mainly to lack of privacy.

The cages which are of interest to research workers are of two types, nam the breeding-cage and the flight cage. A flight cage can be of any size and conveniently made by combining a number of breeding-cages.

Breeding-cage. The best results in breeding canaries are obtained by the ating of one cock to one hen. Many breeders consider that it is satisfactory r one cock to serve two hens, but experience indicates that this is not the st arrangement. The factors which govern the specification of a breeding-ge are:

(*a*) The provision of an adequate amount of room.
(*b*) Simplicity of design, to facilitate cleaning.
(*c*) Correct dimensions and proper placing of perches.
(*d*) Convenience of drinking-troughs, feeding-trays and bath.
(*e*) Provision of a partition with its accessories to cater for the pre-mating period.

Size and design of cage. Fig. 54 shows a suitable breeding-cage which can adily be purchased or made. Its dimensions are 40 in. × 12 in. × 18 in.

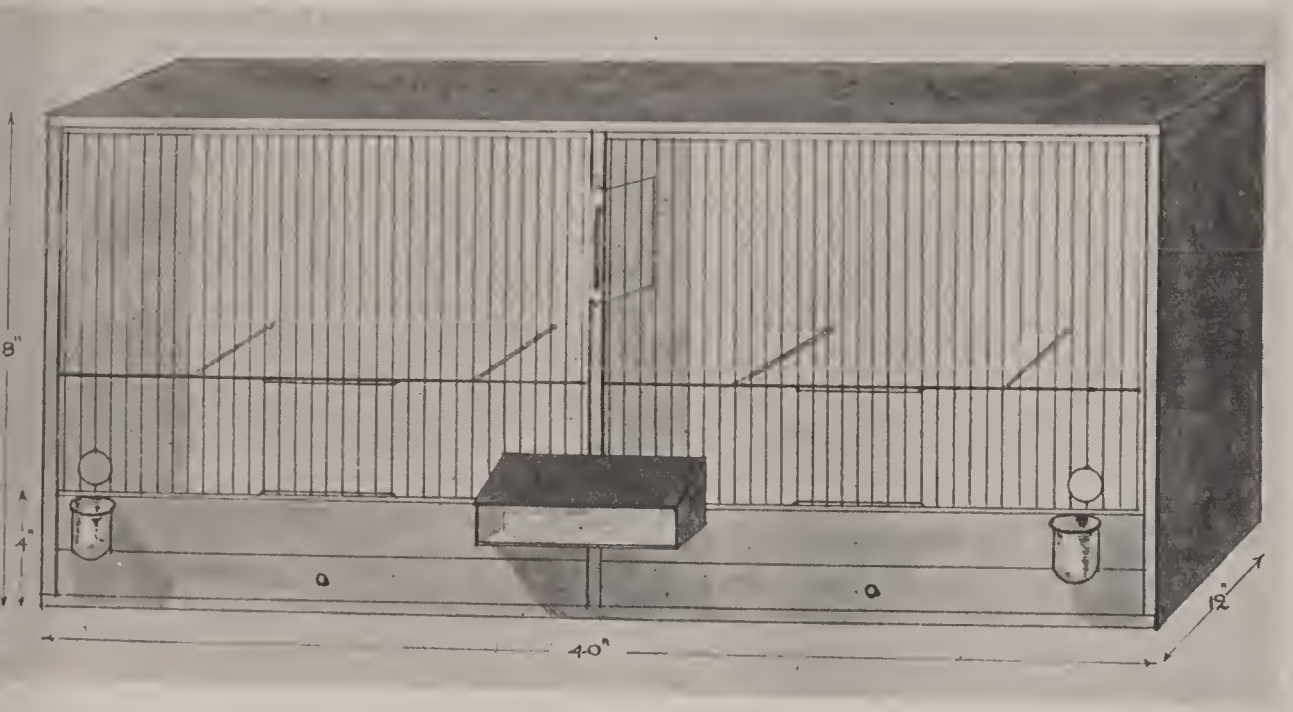


FIG. 54.—CANARY BREEDING-CAGE.

s constructed of wood on all sides except the front, which consists of vertical es with horizontal stays. By means of a movable partition the cage can be ided into two parts, so that the cock and hen can be separate, the partition ng fitted with a removable section which can be replaced by a small wire me; these arrangements permit of the development of the normal courtship ich occurs before the actual mating.

Perches. The construction of the perches closely affects the comfort of the l, and this in turn affects its condition. A suitable perch is shown in . 55 (*b*) and should stretch from the front to the back of the cage, being ached to the wire by means of a groove and to the back of the cage by letting mall nail into the end of the perch and attaching it in dart fashion, or by ans of a hook. The perches should be quite firm, as loose perches make ing difficult and result in unfertile eggs. Three or four perches are required, at an upper level. The sectional dimensions should be $\frac{5}{8}$ in. × $\frac{3}{4}$ in. for

the larger canaries (as Norwich and Yorkshire) and $\frac{1}{2}$ in. × $\frac{3}{4}$ in. for the small birds (Border). A perch of the type shown in Fig. 55 (*b*) is easy to clean b scrubbing and has no awkward corners where parasites may be harboured.

Feeding and drinking utensils. Vessels used for feeding and drinking shoul be as simple as possible in construction to permit of easy cleaning. Glas drinking-vessels are usually the best. Fig. 55 (*c*) shows a suitable seed troug which is readily procurable in bird stores. Feeding-vessels are usually made tinplate painted outside; they should not be painted inside in view of th tendency of the birds to peck the paint. It is said that birds doing so are liab to relapse into a premature moult.

Flight cages. Flight cages can be of any size, according to the size of th aviary concerned. They are used for unflighted birds (i.e. birds that have n reached maturity) and for older non-breeding birds. It is inadvisable to kee young and old in the same flight, since mature and older cocks are liable t molest the young birds.

Typical flight cages consist of a series of breeding-cages fitted with movab partitions. To convert into double breeding-cages, it is merely necessary t insert partitions. The number of perches is governed by the size of the fligh but their structure is the same as that recommended in breeding-cages. Th drinking and feeding utensils are the same in both cases.

Nesting-boxes. These are made of wood (Fig. 55 (*d*)). Some breede use a perforated metal bottom, but a solid wooden bottom is probably pr ferable, since birds are apt to damage their claws in the perforations whe building nests.

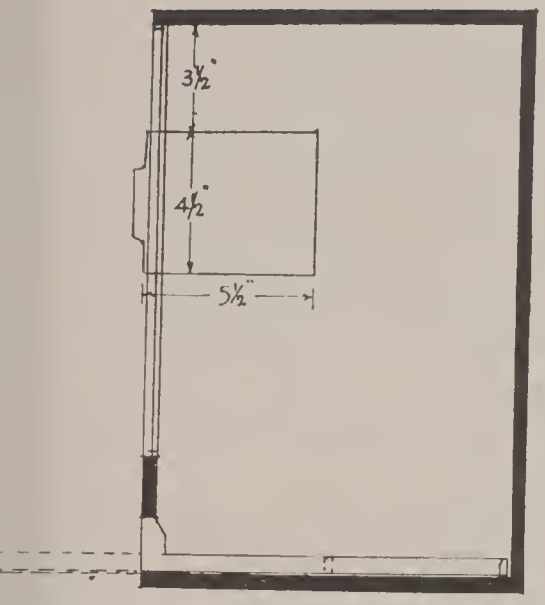
4. Nutrition

In the feeding of canaries it should be remembered not only that the foo has to be suitable in its nutritional qualities, but also that it must be readil taken by canaries. Seeds can be disliked for their flavour or for mechanic reasons—the husk may be too hard to be cracked conveniently by the bir Seeds such as red millet will be refused under normal conditions and will on be eaten to avoid starvation. The basal seeds are canary seed and rape see The accompanying table gives an analysis of food values.

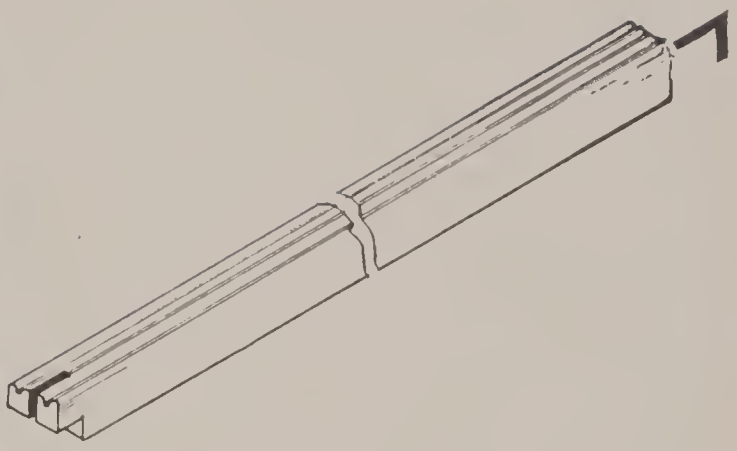
Canary seed is the seed of canary grass (*Phalaris canariensis*) and is grown various parts of the world, including Sweden, Germany, Spain and Sout America. It is essentially starchy and provides most of the energy-food of th canary. Seeds from the different sources vary both in their appearance and their acceptibility to the average bird.

Rape seed is the seed of a cruciferous plant and is similar in appearance t brown mustard seed. Cruciferous seeds are characterized by high fat an protein contents and relatively low carbohydrate. Rape seeds vary con siderably in composition. Some varieties, particularly Indian rape, are hot t the taste, owing to the presence of a glucoside which, when moistened, produc small quantities of a mustard oil. This type of rape is known among breede as "hot rape" and is not suitable for canaries. If given regularly it is liab to send the birds into a soft moult.

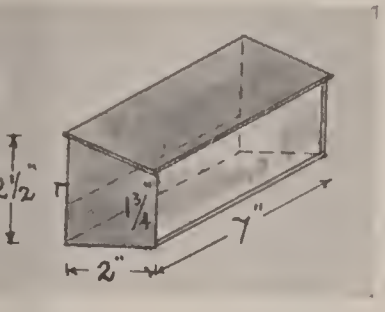
The quality of rape suitable for canaries is the so-called cold rape whic does not produce significant quantities of mustard oil. Breeders usuall distinguish accurately between the two qualities of rape by the simple metho of cracking and tasting them. The best quality, and incidentally the deares



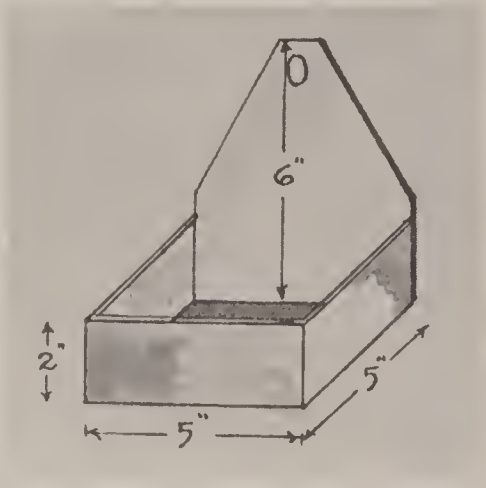
(A) Section through Cage.



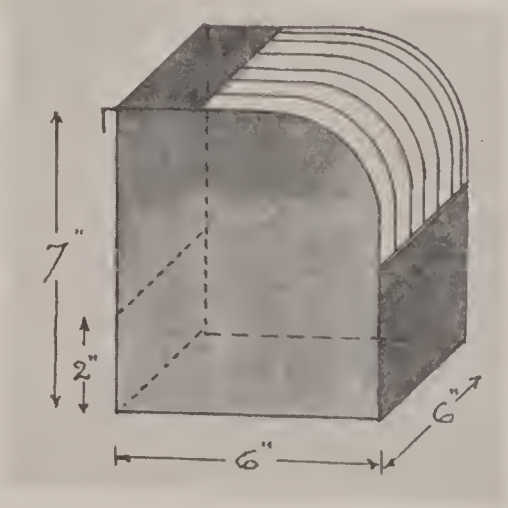
(B) Perch.



(C) Seed Trough.



(D) Nesting-Box with Plain Wood Bottom.



(E) Bird Bath.

Fig. 55.—Details of Canary Breeding-Cage illustrated in Fig. 54.
H. T. Dunham, L.R.I.B.A., Chartered Architect-Surveyor, Norwich, February 1945.)

is the so-called German summer rape or Rübsen rape. This is a relatively bol seed and is much in demand by eminent breeders.

Millet seed (various species of *Panicum*). Opinion varies as to the usefu ness of millet seed for canaries. On nutritional grounds it should be a subst tute for canary seed. In practice, however, it is found that canaries generall refuse to eat it, with the exception of the yellow variety, and much prefe canary seed. There are three varieties : yellow, white, and red.

Canary, rape and millet are to be regarded as basal seeds. A suitable com bination is 2 parts by weight of canary, with or without a small proportion millet, to 1 part of rape.

The seeds should be free from rancidity. Not only has a canary a kee sense of selection for fresh seeds, but also if it eats rancid seeds it is liable t suffer from inflammation of the digestive organs, which may lead to death.

Other seeds. Certain seeds are used for limited periods to treat speci conditions. Hemp seed, which is a fatty seed, is usually given once a wee during the winter months to provide extra fat. Maw or poppy seed is give as a tonic and appears to act by regulating the bowels. Linseed (flax seed) given particularly to birds suffering from premature or soft moult. Niger see is added to the diet in small amounts at the time of breeding.

These special seeds should not, however, be given for prolonged periods they are to be regarded as corrective rather than " bread-and-butter " seeds.

ANALYSES OF BIRD SEEDS.

Seed.	Ash.	Protein.	Fat.	Fibre.	Carbohydrate
Canary	7·0	16·7	6·8	7·6	61 9
Millet	4·0	12·5	4·1	9·0	70·4
Rape	6·1	24·7	42·6	4·4	12·0
Oats	3·5	12·1	5·3	12·1	53·8
Wheat	1·8	12·3	1·7	2·5	81·7
Barley	2·7	11·1	2·2	5·3	78·7
Linseed	3·9	24·2	36·5	5·5	30·0

The seed hopper, washed weekly, is kept filled with the canary-rape mixtur Each morning the loose husks are blown away and the seed is left in the hoppe which is kept replenished. Birds require a source of calcium and this is usuall given by inserting a piece of cuttlebone between the wires of the cage. Som ground eggshell or oystershell should be added to the gravel at the bottom the cage.

Water is changed daily.

Greenstuff. Once or twice a week it is necessary to give birds green food i the form of chickweed or lettuce leaves. It is usual merely to fix the green foo to the wires of the cage and allow the birds to take what they wish. Wheth the consumption of green food is necessary for regulating the normal excretor processes, or whether its function is to provide essential vitamin A or C, or t keep body fluids in a sufficiently alkaline state, is not known with certainty.

5. Breeding

Breeding is usually started late in March or early in April. A good criterio is the climatic state. It should not begin until the thermometer is in th neighbourhood of 50° F. The pairs should be selected earlier than this an

nsferred to the breeding-cages, with the cock and hen separated by the solid rtition. By the middle of February a wire partition should be substituted order that the birds may see one another. A little niger seed should be ded to the diet twice a week and in early March hard-boiled egg mashed up th breadcrumbs or biscuit food should be given. Finely-ground eggshell or stershell should be sprinkled on the floor with the gravel, which should nsist of washed sand not too fine but free from dust.

When the birds begin to feed each other through the bars they are ready mating. The wire slip is withdrawn and the birds allowed to meet. The sting-box can then be inserted, and nesting-materials (deer-hair or cow-hair l moss) should be placed in the cage.

As the first three eggs are laid they are taken each in turn from the nest l replaced by dummy eggs. The eggs are placed in a box at room-tempera-e. In the afternoon of the third day (usually one egg is laid daily) the three s are returned to the nest in place of the dummies. The reason for this cedure is to ensure that all eggs shall be hatched on the same day. The cock hen again separated by the wire slip from the hen, who is allowed to sit on clutch of eggs. On the thirteenth day a little egg food (hard-boiled egg ed with breadcrumbs and moistened) is given to both birds, and once ching has begun (about the fourteenth day) egg-food, or one of the proprietary tling foods, must be given three times a day at 4-hourly intervals.

After the chicks have been hatched eight or nine days and their eyes are n the cock may be allowed to join the hen, but if the hen neglects to feed chicks it may be necessary to remove the cock again. The chicks are fed n egg and bread-crumbs, with a little cracked canary seed when they are e to feed themselves.

When they are sixteen days old the hen will probably wish to go to nest n—her second round. A fresh nest and nesting materials are provided, the technique of temporarily withdrawing eggs, etc., is repeated. Three our rounds can be hatched in a season.

A daily inspection must be carried out for dead chicks, which must be oved at once.

6. Cleaning

The cages should be thoroughly scrubbed and lime-washed annually. ry month they should be washed with soapy water and sprayed with a nfectant solution. Every day the movable bottom of the cage should be oved and scraped free of excreta and discarded husks, seeds, etc. These ning operations are essential to prevent the development of red mite, a site which will attach itself to a bird and cause it to peck away its feathers, result of irritation, until it becomes almost bald.

mmediately before the breeding season the birds are usually dusted lightly a pyrethrum powder (e.g. Keating's powder). To ensure that the der comes into contact with the skin the feathers are gently blown during dusting.

Bird baths (Fig. 55 (*e*)) are placed in position at least three times weekly.

7. Moulting

Normal moulting takes place after the end of the breeding season, in the ths of July and August in Great Britain. During the moulting period the

bird is in its least resistant state and care is particularly necessary to preve chills. Plenty of fresh air and sunshine are needed, but baths should be giv only on the warmer days. Once or twice a week a tonic may with benefit given, such as a crystal of ferrous sulphate about the size of a pea added to t drinking-water.

The signs of moulting are apparent drowsiness followed by the appearan of a few loose feathers on the bottom of the cage.

8. Colour Feeding

Colour feeding of canaries is widely practised by breeders to increase t value of the birds. The essence of the process is to administer a red-colour food when the feathers are forming (i.e. during the moulting period). T red colour reaches the yellow feathers and results in the production of an oran bird. It is provided by ground paprika of good quality mixed with ha boiled egg, bread crumbs, and a little vegetable oil. A little of this is giv each morning during the moulting period. Up to the present, the only sat factory colouring ingredient has been found to be paprika. Red oil-solul colours in general have not proved satisfactory. Further, if the colour paprika is extracted and fed separately, unsatisfactory results are obtain The process at the present time must be regarded as empirical, and the reas why red oil-soluble colours, other than paprika, are unsatisfactory remains the moment unexplained.

9. Diseases

Infectious diseases of canaries. In an extensive communication deali with the diseases of cage and aviary birds, Henry Gray (1936) stressed the f that infection generally is introduced by birds coming from an infected sto bird-room or aviary or by exhibition birds returning from a show. He dr attention to " the despicable practice of some fanciers, who, when they hav dangerous disease in their stock, rapidly dispose of apparently sound stock dealers who in turn dispose of the birds to various customers " ; he points that birds which have survived an outbreak of infectious disease may be carri but may remain healthy until subjected to the strains of mating, moulting crowding together. As a measure of control, he advises that all newly acqui birds should be kept in isolation in clean disinfected cages for at least a mo before they are allowed in contact with other birds.

Gray's communication should be consulted for advice on the feeding a hygiene of cage birds, for an extremely good review of the common infecti and non-infectious diseases and for a useful bibliography. Brief mention m be made here of the conditions which Gray regarded as common and importa

Infectious necrosis of the liver and spleen (bird fever, paracholera, pseu tuberculosis). Highly infectious to many birds ; the causal organi *Pasteurella pseudo-tuberculosis rodentium* is also pathogenic to many mamm especially rodents. The period of incubation is from 3 to 5 days, the signs disease indefinite ; death may occur within a week. On post-mortem exami tion, characteristic nodules are found in the spleen and liver. The diagn should be confirmed by isolation and identification of the causal organism.

Paratyphoid (" typhoid," " contagious enteritis," " septic fever Affected birds are dull, with ruffled feathers and diarrhœa ; death may oc

ddenly after convulsions. On post-mortem examination the liver and leen may be found to be enlarged and the intestines inflamed.

Gray (1936) considers that *Salmonella pullorum*, the causal organism of acillary White Diarrhœa or Pullorum Disease of fowls, and *Salmonella llinarum*, the causal organism of Fowl Typhoid, are common causes of this ndition in canaries ; he suggests that the source of infection may be eggs om infected hens and foodstuff made from diseased carcases. Another ssible source is " egg-flake," a by-product of calico-printing and used in the eparation of biscuit meal.

Villani (1937) has found that the canary is susceptible to infection with *pullorum*.

Another salmonella organism found causing paratyphoid in canaries is *aertrycke* (*S. pestiscaviæ, S. typhi-murium*), Beaudette & Edwards (1926) d Emmel & Stafseth (1929). Fenstermacher *et al.* (1944) have described tbreaks and have demonstrated the presence of this organism in affected rds. Diagnosis should be confirmed by bacteriological examination but it ould be remembered that salmonella are frequent secondary invaders in ittacine birds infected with the virus disease, psittacosis, which has been und to have a wide range of pathogenicity for birds, including canaries. If ere is any history of proximity to parrots, budgerigars or other psittacine rds, the laboratory examinations necessary for the diagnosis of psittacosis ould be made.

Paratyphoid-like infections with coliform organisms have been described.

Streptococcosis was described by Ruhling (1925) and Voker (1926) and shown be caused by a streptococcus probably transmitted by the red mite, *Dermnyssus gallinæ*. Affected birds are dull and sleepy, have diarrhœa and fficulty in breathing ; they wheeze and squeak continuously. On post-rtem examination, foci of inflammation and necrosis are found in the liver, gs, spleen and other organs ; the spleen and liver may be swollen and there y be sero-fibrinous exudate in the thoracic and abdominal air-sacs.

Aspergillosis, infection of the lungs and air-sacs with a mould or fungus, *pergillus fumigatus*, occurs in all species of birds and is quite common in naries (de Jong, 1912). According to Gray (1936) an acute type may occur sing " infectious pneumonia " which may appear and terminate fatally hin 8 to 12 hours. The less acute or more chronic type is more common, signs being those of difficulty in breathing with wheezing, gurgling and tling noises, somnolence, inappetence, thirst and pyrexia. Slightly affected ds become dull, weak and emaciated. On post-mortem examination there found whitish-yellow crusty coatings on the tongue, palate, larynx, trachea l syrinx, caseous foci in the lungs and on the air-sacs.

Diagnosis can be established by microscopical or cultural demonstration of fungus.

Prevention is by avoiding the use of mouldy food or litter. Gray advocates t birds should be kept in clean cages and allowed exposure to direct sunlight l fresh air.

Tuberculosis in the canary is rare, although this species is quite suscep-e.

Avian pox in canaries is not uncommon. It has been described by Eberbeck Kayser (1932), Reis & Norbrega (1937), Grosso & Prieto (1939), Antoniotto Romat (1940). In canaries used for experimental transmission of bird

malaria, Kikuth & Gollub (1932) encountered an unusual type of virus whic Burnet (1933) investigated and showed to be a variant of avian pox.

According to Lahaye (1930), (*a*) canary or passerine pox is transmissible t canaries, finches and sparrows but not to pigeons nor to fowls ; (*b*) pigeon po is transmissible to pigeons and fowls but not to canaries and passerine birds (*c*) fowl pox is transmissible to fowls but not to pigeons nor to canaries an passerine birds. Irons (1934), on the other hand, found one strain of pigeo pox which was transmissible to canaries ; Reis & Norbrega (1937) described strain of canary pox which could infect chicks and pigeons as well as passeri birds. The strains of canary pox examined by Grosso & Prieto (1939 Antoniotto & Romat (1940) and Burnet (1933) were found to be pathogen only for canaries and passerine birds.

The disease in canaries is usually rapidly fatal ; in the outbreak described b Reis & Norbrega (1937) the mortality was 98 per cent. Three forms may l seen : (1) wart-like lesions on the comb, wattle and face ; (2) diphtherit lesions on the membrane of the mouth ; (3) localization in the nasal chambe (roup). Diagnosis is usually made on the appearance of the characterits lesions but if there is doubt, sections of tissue may be cut and stained f inclusion bodies or transmission experiments carried out on suitable birds.

Control is by destroying any affected birds at once and by segregati susceptible birds from contact with newly purchased and wild birds whi may carry the infection.

Reis & Norbrega (1937) successfully immunized canaries with a live vacci made from the infecting strain.

Psittacosis (Ornithosis). According to Meyer (1943) canaries and vario other species of the order Passeriformes contract psittacosis when exposed cages or rooms where infected parrots or parrakeets are kept. The period incubation varies from 15 to 60 days and illness lasts from 3 to 20 days ; t birds appear listless and huddle with ruffled plumage in one corner of the cag Death may occur suddenly.

On post-mortem examination, the vent is found to be soiled with greeni or greyish fæcal concretions and the subcutaneous tissues and serous linin jaundiced. The liver is enlarged, fatty, irregular, mottled and sometim studded with small foci of necrosis. The spleen is always enlarged, soft a sausage-like (from 15 to 18 mm. long and 4 to 8 mm. thick) ; the kidneys a swollen, greyish and soft ; urine accumulates in the cloaca ; the serous lini of the sternum, the air-sacs, pericardium and liver are frequently covered by plastic purulent exudate. The intestines are infected and full of liqu chyme. In long-standing cases, focal pneumonic areas are found in the lungs

Diagnosis. As the virus of psittacosis is pathogenic to man, great ca should be exercised in examining suspected birds. A special gown, rubb gloves and a suitable face mask with goggles are recommended. Films of t spleen, liver, etc., may be made and stained by Giemsa or other suitable stai the intracellular and extracellular virus bodies (L.C.L. bodies or *Microbacteri multiforme*) are sought for under the microscope. If microscopical examinati is negative, pieces of liver, spleen and kidney should be ground and suspend in broth ; the suspension may require filtering through sand and should inoculated into four mice intraperitoneally or into rice-birds. Meyer (194 states that mice inoculated intraperitoneally with virus from birds may sh at autopsy merely an enlarged spleen in which the virus particles are usua

ery scanty ; if a suspension of such spleen is inoculated intracerebrally into ice, a chorio-meningitis with paralysis is produced ; films of the choroid exus suitably stained usually show an enormous number of virus bodies.

The complement fixation test, according to Meyer (1943), is most useful in e detection of psittacosis in an aviary. Sera from infected birds may be rongly (1 : 128 ++++) to moderately (1 : 2 to 1 : 4 ++++) positive. owever, as it is impossible to obtain sufficient blood for the test from small rds, the complement fixation test is merely supplementary to the usual croscopical examination and inoculation of mice.

Specific Ophthalmitis. Gray (1936) states that this may occur as an enzootic aviaries, the condition beginning as a conjunctivitis with a watery discharge hich later becomes sero-mucous ; pan-ophthalmitis ensues and the affected rds become blind but their general health is not affected. Gray suggests at the cause may be a virus transmitted by some vector.

Cataract appears in certain in-bred strains of canaries.

Alopecia and **feather cysts** or " Lumps " (" Hypopteronosis cystica " ; re, 1931) are also hereditable in nature. Other abnormal conditions scribed by Gray are **uropygitis** or " old gland disease," **overgrowth of the k, atrophy of the beak, dry gangrene of the feet, sore feet, slip toe, osteo-lacia, rickets,** " megrims " or **labyrinthine vertigo, " gout," coccidiosis,** used by the protozoan *Isospora lacazi,* parasitism with **capillaria worms,** estation with various feather and skin **mites** (" scabies "), infestation with red mite *Dermanyssus avium.* For advice on the methods of prevention d treatment, Gray's series of articles should be consulted.

General instructions on the control of ecto- and endo-parasites are given in apter 3.

[The following notes on health and disease have been contributed by Drs. ran and Edgar :

Excreta. An important routine observation which gives evidence of dis-er is the nature of the excreta. In health they are black and white, and ned. If they are green and offensive it is a sign of inflammation which may due to a variety of causes, faulty diet being a frequent one. Different eders have their own personal remedies, but a satisfactory treatment is to lace the mixed seed diet by one of plain canary seed, and to add small ntities of Epsom salts to the water. At the same time the bird should be en milk sop (bread soaked in warm milk and water).

Loose excreta are frequently a sign of **chill on the liver.** A drop of whisky he water will help, or 3 drops of sweet spirits of nitre with 5 drops of glycerine, given in the drinking-water.

Asthma or wheeziness is a common result of using dusty seed. The nostrils uld be bathed with cotton-wool and warm water. Spirits of nitre and cerine may also be given as above.

Red mite is a common nuisance. Its presence is indicated by the bird stantly ruffling up its feathers and pecking them. The mites spend the in crevices of the cage, coming out at night to attack the bird and causing o lose sleep. The remedy is to brush paraffin into all crevices and on the s of the perches, while the bird should be dusted with Keating's powder or milar insecticide. This should be well rubbed in under the wings and over back and belly.

Sore eyes are best treated by bathing the eyes with tepid boric acid.

Soft moult, or moulting out of season, has various causes. Excessiv warmth is a common one, while shock brought about by sudden switching-o of lights at night may frequently have the same effect. Milk sop is useful, an half a teaspoonful of whole linseed added to the seed mixture. Later an iro tonic should be given, preferably a crystal of ferrous sulphate about the siz of a pea added to the drinking-water one day a week.—*Ed.*]

[*Editorial Note.*—Bishop (1942, pp. 10–11), stressing the importance of standa conditions in testing the anti-malarial activity of compounds, writes :

" Several laboratories have experienced difficulty in keeping canaries healthy whe large numbers are involved, and their work has been interrupted by the outbreak epidemics. In the Molteno Institute [University of Cambridge] the birds are kept a separate room, and the windows and doors are screened to prevent the entrance mosquitoes. Unpainted metal cages of a simple design, which are easy to clean, a used. The cages are arranged on metal tubular shelving which avoids the accumul tion of dust, and of fæces and seeds cast out of the cages. By this method lar numbers of cages can be stocked in a moderate-sized room. The birds are kept individual cages which are cleaned frequently, and always sterilized before re-us The cages are kerosened along all cracks at intervals of a few weeks, in order to contr red mite which attacks cage birds. A balance diet is given, with green food twice week. The bird-room is kept scrupulously clean, all dust, waste seed, etc., bei removed from the floor daily by a vacuum cleaner. To prevent the introduction disease into the stock new birds are isolated in a separate room, in separate cages, f at least a fortnight, after which only those which appear healthy are admitted to th stock. Canaries have been kept in this Institute in large numbers since 1928, and n outbreak of disease has occurred amongst the stock, though disease has broken o amongst new arrivals during their period of isolation."

" No mosquito work is done in the bird-room, birds required for experiments bei removed to another room."]

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CHAPTER 20

THE KEEPING OF AMPHIBIA FOR LABORATORY PURPOSES

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1. Frogs. 2. Toads. 3. Tadpoles of the frog and toad. 4. Salamander Larvæ. 5. Newts. 6. Axolotls. 7. Anæsthesia and euthanasia.

1. Frogs

Long-term maintenance. The essential conditions for keeping frogs healthy are cleanliness and moisture ; a moderately low temperature also is advisable. Recent experience has shown the great importance of avoiding water contaminated with zinc or other metals (Clark). The main considerations are, therefore, the nature of the containers, the water supply, and the facilities for cleaning. Feeding is described separately.

Sink methods. When sink methods are used a continuous supply of aerated clean water is essential. The outlet of a large sink is closed with a cork through which runs a glass tube to take off the overflow. A dripping tap renews and aerates the water. A platform must be provided for the frogs (Smyth) ; this may be fixed, or a floating piece of wood or cork.

Alternatively the sink may be empty ; the plug is removed and a slight trickle of water from the tap provides sufficient moisture to keep the animals in good health. The open sink outlet acts also as a guard against flooding. Should the outlet become clogged with foreign matter or with frogs (they have a habit of sitting on the drain-pipe) then the overflow will carry away surplus water. Frogs must not be forced to swim in water since constant swimming leads to exhaustion. The empty sink is quickly cleaned by flushing of the tap. The sink is covered with a close-fitting wooden frame to which perforated zinc is fastened. In order to ensure that the water shall not be contaminated by the zinc, a piece of rubber tubing is passed through the latter and connected to the water-tap (Clark). By this method frogs have been kept for two years with a very low mortality rate.

Whatever kind of container is used a firm cover of some kind is essential. This may be made of perforated zinc or wire netting fixed to a frame or bent down at the corners, strips of board slightly separated to allow aeration and weighed down by bricks, or strong open muslin weighted round the edges.

The suitability of tap water for any kind of laboratory animal is a major question which will no doubt be dealt with in due course. Contamination from lead pipes and chlorination are two obvious sources of trouble. The conservation and use of rain-water might be borne in mind. [See also p. 262—*Ed.*]

Temperature is of importance. During the summer months the animals should be kept in a cool place and in the shade. In winter they should be kept at normal laboratory temperature (e.g. 55° to 66° F.). They show no inclination to hibernate, and feed as readily in winter as in summer. If they are

allowed to remain at a temperature much lower than 10° C. there is a danger of their entering the winter stupor or hibernation, from which few recover.

Froggery. Roaf describes the use of a disused greenhouse as a froggery. Before the war 3,000 frogs were obtained from Holland each autumn. The methods of maintenance and feeding worked out were sufficiently satisfactory to allow this supply to last for a year.

Concrete work was built on the floor of the greenhouse. At the far end was a pool 6 in. deep, with a shelving lip. There was a central gangway, surrounded by a 3-ft. wall, which allowed attendants to reach the frogs. On either side of the gangway the concrete floor sloped gently towards the lip of the pool. Any water overflowing from the pool kept the surface of the alleyways moist. At the lower end was a drain-hole protected by wire netting or perforated zinc, to prevent frogs from escaping or blocking up the drain-hole. The pool and side alleys were cleaned at regular intervals, and the froggery was inspected each morning and any dead frogs removed. It is suggested that if an opaque roof had been substituted for the glass one even better results would have been obtained. Boards supported on bricks were used both on the alleyways and in the pond so that the frogs could get into the shade if they wished.

An area or compound under semi-natural conditions may be arranged in some situations, e.g. in a garden. Here the frogs themselves obtain some of their food and are given a supplementary diet of worms and slugs.

Short-term maintenance (up to 10 days). It is clear that the methods already given are the most efficient. If, however, a large stock of frogs must be housed and sinks are scarce, the nearest substitute must be improvised.

Frogs may be put into wooden tubs, glass tanks, portable porcelain sinks, or large vessels of earthenware, in a cool store or area. A firm cover that admits air must be arranged. Twice daily the frogs must be watered from a watering-can fitted with a rose until they are nearly covered with water. The water is then drained away by tilting the container sideways and the frogs are again lightly sprinkled to keep them moist until their next wash. This method provides partly-aerated water and prevents toxic accumulation.

Methods to be avoided are keeping frogs in a few inches of water which soon becomes fouled, and keeping them in damp moss or leaves. The latter method accounts for much mortality, since the litter collects and holds the excreta, and itself decomposes, both factors leading to disease and semi-poisoning of the frogs. If damp moss or the like is used, then it must be changed twice a week and the frogs' container thoroughly rinsed out at the same time. Rough turves, watered regularly, are an improvement on moss, on account of the earth they bring with them and the living grass. Turves can be easily lifted out and replaced weekly.

Whatever method is used, frogs should be inspected daily, and dead ones removed at once.

Nutrition. Frogs can survive for a considerable time without food, but such treatment causes unnecessary suffering and is unpractical. In a weak and starving condition frogs make poor dissection material, and for physiological experiments they require to be in good condition. "Our experience is that muscle-nerve preparations are more reliable when made from well-fed animals" (Roaf). Frogs kept in stock for use from time to time should be fed regularly from the first, particularly as they may have been without food for some time

before arrival. They take food more readily when they are in good condition. Feeding methods which have proved successful and have been adopted by various workers are as follows. [See also p. 262—*Ed.*]

Voluntary feeding. For frogs kept in the greenhouse froggery, maggots obtained from the hide market and mealworms were scattered on the concrete alleyways. The frogs rapidly disposed of these. Sometimes a supply of maggots was bred by hanging up a cod's head or sheep's head in the froggery, so that flies could lay eggs in the flesh. When the maggots developed they fell out and were eaten. Alternatively the heads were kept elsewhere and the maggots were shaken out and later taken to the froggery (Roaf).

Blowfly larvæ (the " gentles " of bait shops) can be obtained in quantities from dealers and fed to the frogs. Mealworms are available from the same sources. Blowfly larvæ can be left in a box of sawdust to pupate, and the box put with the frogs. As the flies emerge the frogs catch them.

Once a week a few frogs may be placed in a small container overnight with a supply of food, such as blowfly larvæ, mealworms, small slugs and earthworms. Four frogs, for example, can be placed in a large biscuit tin with perforated lid. Two frogs can be put in a breffit, i.e. large glass jar, covered with open muslin held on by a rubber band. In this case the groove between the base and side of the breffit must be filled in with wax or plasticene, for otherwise worms and larvæ will lie in it and escape being eaten. A fair allowance for one frog is one 2-in. worm, two slugs or four larvæ. A rotation system can easily be worked out so that all the frogs are fed in turn (Stephenson & von Wyss).

A supply of small earthworms keeps well in leaf mould a few inches deep kept moist, and well covered at night to avoid losing the stock.

Small slugs can be gathered in quantity at dusk, especially in damp weather from a lawn, the edge of a road with grassy verge, or the general surface of a flower bed. The slugs can be stored in tins with perforations in the lid. In a cool place a supply will keep for weeks. White or grey slugs are much preferred as food.

Forcible feeding. Frogs are very easily fed if handled in the following manner. The animal is held lightly in the palm of the left hand with the stomach facing the attendant and the thumb immediately under the frog's forearm. The hind legs are allowed to relax. If the animal is treated with reasonable care it will not attempt to struggle, in fact after being handled once or twice it will not resist. The lower jaw is opened by inserting blunt forceps at the side of the mouth, and the mouth is then kept open with the forefinger of the left hand. A piece of liver about the size of a shilling is placed well back in the mouth behind the eyeballs, and the mouth is closed. If the frog makes a swallowing movement there is little chance of the food being ejected, but food must not be forced down the throat, for this causes eversion of the stomach. The frog in a short time adapts itself to the procedure and suffers no discomfort. At first it may, while being fed, remove the food from its mouth with its hands. By controlling the frog's hands with the middle finger this difficulty is easily overcome. Frogs should be fed in this way about once a fortnight (Clark).

2. Toads

The methods described for keeping frogs can also be used for toads. Toads do not, however, need such damp conditions as frogs. They like to sit in niches

mong stones, and a rough rockery of big stones or bricks should be provided or them in a large airy cage. The rockery should be built on a bed of earth nd gravel kept permanently moist. Toads easily become tame, and if food placed for them on the floor of their cage at about the same time each day ey soon learn to take it without trouble. Non-hairy caterpillars can be added their menu.

3. Tadpoles of the Frog and Toad

Spawn should be placed in water under a dripping tap, or failing this the ater should be changed daily. As soon as the tadpoles hatch they should e removed with a teaspoon or dipping-tube and placed in containers, no jelly eing left with them to encourage moulds.

The best container is a large shallow pie-dish at the bottom of which is aced a layer of pond silt or a quarter of an inch of garden earth and a few nall stones. Ample pond weed should be provided, preferably a mixture ch as elodea, stonewort and milfoil. [Weed can be obtained from : L. Haig, eambrook, Newdigate, Surrey.] The water may remain unchanged unless me accident occurs to make it cloudy, and dishes should be kept in a fairly od light to encourage micro plant growth on their sides. Tadpoles also feed the micro population of the surface film.

When the hind legs are partly grown a small quantity of animal protein ould be provided, e.g. six dice a quarter of an inch across of hard-boiled egg r dish, or small pieces of chopped earthworm. These should be removed en any mould appears on them. If raw meat or liver is used it may be tied string hung over the side of the dish and removed as soon as it has swollen the water or lost colour, usually after about three hours. Dried egg has not en tried but this should be even better than hard-boiled egg, if cooked and t into dice.

Near the time of metamorphosis, sloping stones should be arranged pro- ting above the water. As newly metamorphosed frogs are very liable to mp out, get lost, and die, a suitable cover should be kept over the dishes. tamorphosed frogs can be put into a separate container with moist earth, ss or moss. They do not feed for a few days, and as soon as opportunity rmits they should be taken to the nearest pond or garden. The frog and d population has been seriously depleted during the last few years by favourable seasons, and there is need to conserve the natural fauna.

4. Salamander Larvæ

Females obtained for dissection sometimes give birth to young. More ally, the uteri are found to contain several young during the course of section. It is well, directly the animals have been opened under water, to ove larvæ (still within their vitelline membrane) and transfer to cool, ated water. Many hatch and are quite vigorous.

The larvæ may be reared in the same manner as tadpoles. They develop er more quickly than tadpoles and a culture of *Daphnia*, or any small pond serves very well while the carnivorous habit is developing. An advantage alamander larvæ is that they are large, very tame, and quite ready to take d from forceps. *Chironomus* larvæ, obtained from the mud of most butts, erns or ponds, are very suitable food. If one of these is held to the animal's

mouth with forceps it is taken at once. *Tubifex* and tiny earthworms also ar suitable.

Salamander larvæ are useful also because they are easy to feed after meta morphosis. When they leave the water they should be kept in a large bowl o sink with a floor of damp earth, a few flat stones, and some damp moss, turve and leaves (i.e. the woodland floor). For a few days they will not take food but when internal adjustments are complete they do so readily. The food mus move. Small earthworms were used by the writer, but no doubt young blowfl larvæ would prove as good.

The most simple feeding method is as follows. Take out two or thre larvæ, let them run on the bench, and place food before them. Directly the have fed, put them in a deep container for safety. Feed another batch, an when all are fed, tip them back into their cage. About fifteen minutes a day required for feeding. The earth, moss, etc., must be renewed each week.

The animals are easy to rear and to feed, and are attractive and tam After four years they are of suitable size for dissection. Considering the diff culty there is in obtaining salamanders, it would seem worth while for an laboratory to ensure its own supply in this way.

Adult salamanders need a large airy container with a floor of damp eart and niches among stones, or small flower-pots, in which to shelter, with mos dead leaves and twigs. Twice a week a piece of worm about 3 in. long may b given. The animals need exercise and should be allowed to run about th bench after feeding. Beware, however, of their ability to escape!

5. Newts

Newts should be kept in a large aquarium slightly tilted at one end, with little water at the lower end and a piece of damp turf filling the upper en Some flat stones should be left on the turf as the newts often like to crawl und these. The aquarium should be covered in the same way as for frogs. Sma earthworms, *Chironomus* larvæ, *Tubifex*, small slugs and small blowfly larv are suitable food. Very small newts may be fed on *Enchyræids* (Baker).

6. Axolotls

Axolotls live well at ordinary room-temperature. It is said that th thrive in open-air ponds in this country even through the winter. Whe animals need to be kept separately, each may be placed in a small glass bo covered with butter muslin held down firmly by an elastic band. This necessary since on occasion they take a flying leap from the bowl and soon d if not discovered and replaced. The water must be changed daily.

Axolotls should be hand-fed. Pieces of lean meat or earthworm are su able. They take moving food only, so that pieces of meat must be held forceps and moved slowly to and fro in front of them until they make a sudd dash. Two or three small pieces every other day are sufficient. Axolo will not take more than they require (Hentschel).

Axolotls can be kept in any ordinary tank with a well-fitting cover of pe forated zinc. They do well in water about six inches deep, which allows the to come up readily to breathe. The white forms do not like bright ligh They appreciate a small flower-pot laid on its side in a corner and will ret with head and shoulders in this for hours at a time.

The water should be changed once a week. The animals can be lifted out y hand or with a net and put in a small bowl while their tank is rinsed. The ater of the bowl, and clean water of the tank, should be tested with the hand) avoid any marked difference in temperature. The water can be just tepid, ut causes discomfort if it is warmer. Axolotls can stand quite low tempera- ures. Ice formed on the surface causes them to become torpid, but if the mperature is very slowly raised they resume activity.

An axolotl after feeding for some years on earthworms refused this food ; ossibly setæ had accumulated in the gut. Small white or grey slugs were ien tried with success and the animal was fed on these for several years after- ards. It also ate tadpoles on occasion. The method employed was first to ipe excess slime from the slugs and then tap on the side of the tank to attract e animal's attention. It then stood with raised head and after a moment ually emptied its mouth of air. It was then ready to catch a slug dropped to its nose and often did so with great precision. If it missed, the slug was trieved by putting the end of a wooden ruler under it and drawing it upwards ainst the side of the tank, and the process was repeated. Sometimes the olotl picked up the slug from the bottom of the tank. If the slug crawled ross its path, by depressing its head the axolotl could look at the floor of the nk and at the right moment scoop up the slug with a strong indraught of ater. Drowned slugs were never taken. Three small slugs every other day ere sufficient food.

It is wise, when approaching a tank, to give the animals warning before aking any sudden movement. They may be asleep, though, of course, the es do not close. If startled they dart about the tank in a state of panic ich can hardly be good for them, and refuse food for some time afterwards. gentle tap or so on the tank is enough to rouse them for feeding or water- anging.

7. Anæsthesia and Euthanasia

Anæsthesia. Urethane dissolved in water has been found by many workers be a very convenient, useful and apparently harmless anæsthetic for phibia. It has proved satisfactory in anæsthetizing tadpoles and is useful en the circulation in the web of a frog's foot is to be demonstrated. The g is placed in a beaker of 1-per-cent. to 2-per-cent. urethane solution until conscious (about 5 minutes). The web can then be spread on the stage of microscope in the usual manner. The frog remains still for about 15 nutes, which allows sufficient time for a large class to view the demonstra- n. The frog can then be allowed to recover in clean water.

For axolotls, Hentschel writes : " On the advice of Professor Spaul, of eds, I used a 1-per-cent. urethane solution. I found that the accuracy of solution did not matter very much, and after some experiments I just used lrop a rough approximation of the necessary crystals into the water in which axolotls were living. They take a little time to become unconscious, which be easily judged by touching them to see if there is any reaction. If deep esthesia is required, it is advisable to wait until they float to the surface and n on their backs, though I should say it is unwise to keep them in this dition for long. As the operations I performed could always be done ckly, I can give no information as to how long they can be kept under this esthetic. To bring individuals round again I found it was best to transfer

them to a dish of water and allow the tap to run on to the dish. They soo come round and seem to suffer no ill-effects. I anæsthetized some anima several times and they seemed none the worse for it."

Euthanasia. The following methods are advocated for killing frogs :

(i) The frog may be placed in a tin containing cotton-wool soaked i chloroform or ether.

(ii) The frog may be stunned, and then the spinal cord and brain pithe direct (West). This is done by snicking through the skin ju behind the head, bending the head forward and inserting a seek between the occipital region and the atlas. The seeker should H worked thoroughly to and fro in the brain and in the upper part c the spinal cord.

(iii) As it is not easy to stun a frog, a humane and simple method is t place it for 5 minutes in a beaker containing urethane solutio before pithing. This would give satisfactory material for mo purposes (Stevenson).

(iv) Decapitation is a merciful method when nerve-muscle preparatio are required.

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[*Editorial Note : Living Specimens in the School Laboratory*, the 2nd edition of whic was published in 1944 by the General Biological Supply House, Inc., 761–763, Ea Sixty-ninth Place, Chicago 37, Illinois, contains notes upon Salamanders, Toads (*Bufo* sp Tree Frogs (*Hyla gratiosa* and others), Bullfrogs and other aquatic frogs, " Grassfrogs (*Rana pipiens* and others), and Tadpoles. Useful notes are given also upon the constru tion of aquaria and terraria. A bulletin issued by the same company, *The Effect Thyroid on Tadpoles*, deals with the acceleration of metamorphosis which may be induc in the laboratory. Brief notes upon the environmental and nutritional requirements various species of frog, toad and salamander are given also by Ernest P. Walker (194 pp. 362–363) in his paper " Care of Captive Animals " from the *Rep. Smithson Instn.* 194 pp. 305–366.

Two Soviet papers of which abstracts are in UFAW's possession are :

Predelski, A. A. (1941). Food substitutes for axolotls. *Laboratory Practice*, **5**, 26–27.

Sudarikov, V. E. (1939). Change of water in tanks for aquatic animals. *Ibid.*, 9–1 23–24.]

An important note on **disease control in frogs** is given on page 262.

CHAPTER 21

XENOPUS LÆVIS DAUDIN

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1. Introduction. 2. Habits. 3. Nutrition. 4. Breeding. 5. Handling. 6. Test jars. 7. Determination of the sexes. 8. Diseases.

1. Introduction

The South African claw-footed toad is an amphibian belonging to the group *ossa*. Morphologically it is a close relation of the Pipa toad, and the

Fig. 56.—*Xenopus lævis* Daudin in Copulation.
(Note eggs attached to bottom of tank.)

[Reproduced by the courtesy of the *Aquarist and Pondkeeper*.]

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exclamation " What a strange frog," so often overheard in front of their ta at the reptile house, seems fully justified inasmuch as the Aglossa are ve different in shape and habits from our indigenous toad (Fig. 56).

The Aglossa, as their name signifies, are tongueless, and their eustach tubes unite before opening into the pharynx by a single median stor *Xenopus lævis* D. in particular has no teeth, and its skin shows rows of muc producing glands on the dorsum which render the animal so slippery that i difficult to hold without a net or a towel. On its back it shows a mott pattern composed of brown and black patches, and the animals certai become darker on a dark background than on a light one ; but compared w that of other experts in the field of mimicry, *Xenopus's* power of adaptatio only limited. The belly is white with a tinge of yellow.

As for the " claw foot," this is a misnomer. It is true that the termi phalanx of three of the hind toes looks rather prominently back, but these t are quite soft and their only function, so far as the writer has observed, is help the animal to get rid of its discarded skin when the time for shedding has come.

The most interesting features of these toads are those concerning th natural habits and their development, and as these have a very definite bear on their value as laboratory animals and their husbandry in captivity they have to be further described.

While *Xenopus* can, of course, be used for all those experiments commo carried out on frogs, it has been found particularly useful in investigations the influence of the pituitary gland on colour changes, the study of the influe of many substances on the gonads and, particularly, in pregnancy tests.

2. Habits

So far as it can be pieced together from the observations of authors w studied *Xenopus* in their natural surroundings, the annual life-cycle run follows. These toads live in the ponds and swamps which the South Afric call " vleis." Some of these " vleis " are brackish, some not ; some are p manent with deep clear water, some temporary, shallow and muddy. Som the latter have connections with permanent water, some are separated fro by many miles of desert. Rainfall in May calls forth the toads from their hi places, and copulation with spawning has been observed from May to Aug In captivity one female has laid up to 15,000 eggs (Bles, 1906, *a*, *b*). Tadp are found in ponds of all sizes and descriptions until October, when they und metamorphosis. Those young toads which are hatched in permanent po remain there until the next spring, but those hatched in shallow water wh entirely dries up later on dig themselves into the soil until the next rainy sea (Rose, 1924–26). The statement that *Xenopus* never leaves the water (Bre 1912) must, therefore, be qualified in those cases in which the water leaves toad. In a few cases mass treks from dying pools to permanent water h been observed.

It is extremely fortunate for the biologist with little laboratory space no elaborate aqua-terrarium that *Xenopus* has taken to living entirely in water. Here the eggs hatch, the tadpoles grow up, the metamorphosis ta place, and the young toads emerge and grow in about three years to their size. The animals are, therefore, amphibian only in so far as, in the growi

ge, they breathe through lungs, but ordinarily they do not leave the water ept for an hour's excursion to a wet stone on which to enjoy a rainy shower. sunny days they lie sprawling and motionless just below the surface, their ies hidden by a leaf floating and their nostrils just raised above the surface llow them to breathe. This way of life allows us to keep *Xenopus* in tanks quaria very much after the manner of fish, and so greatly facilitates the nagement of a *Xenopus* laboratory. An animal which accidentally gets out ne tank and cannot find its way back soon dries up and dies, and any tests xperiments must be carried out so that the toad remains covered with er throughout. The most congenial temperature for these toads is 24° C. F.), but they tolerate variations from 10° to 30° C. On the whole, although come from a sub-tropical country and have even been found in the ical parts of Africa, these toads tolerate excessive cold better than excessive , particularly when confined in great numbers to small containers. Con- ing the hardships they have to tolerate on their long journey from Cape n to London they appear to be hardy enough. In peace-time the average of animals on the journey was never more, and often less, than 5 per cent. has not entirely interrupted these frog imports. Losses, not through ny action but through lack of care in handling the vulnerable cargo, have few occasions risen to 100 per cent., but other consignments have reached country with no more than a peace-time mortality.

3. Nutrition

he question that became most acute during the war and is still exercising ngenuity of the laboratory staff is that of feeding. Unfortunately *Xenopus* tirely carnivorous and although its food need not, like that of many other nibians, consist of live animals, it is at present not easy to provide the unt of protein required to feed a thousand lodgers which are ravenous at mes of the day or night. Things might be easier if these toads had a r alimentary canal. As it is they evacuate their half-digested food within hours of feeding, and it would be surprising to find a specimen not ready k for more than it is ever given. Minced lean beef, horseflesh or liver was hoice in pre-war days. Fat is rejected. Minced fish, raw or boiled, has found useful, and earthworms arouse great excitement in the tank. n, on rare occasions, frog tadpoles and small newts are available they are ted with glee, and *Enchytrea*, *Daphnia* or *Cyclops* would no doubt be suit- f they could be obtained, but this short list exhausts all likely substitutes. pound of minced meat per week for five hundred frogs is sufficient even laboratory conditions where frequent ovulations are induced. An ional fasting week does not affect the animal's health. To ensure even bution in the tank the minced meat is stirred up with water and poured in. morsel is gobbled up within a few minutes. Earthworms can be given nd, and the toads become quite tame in the process of feeding.

ne short and incomplete digestion of *Xenopus* has already been mentioned. act that, twenty-four hours after a feed, the water in the tank becomes nely polluted, and in the summer smelly, indicates that provision must ade for frequent and complete changing of the water and washing of the , these manipulations to involve as little time and trouble as possible. k which the writer had made in 1938 with these requirements in mind

has fulfilled its purpose so well that a description of it is given here (Fig. 5
The tank is made of galvanized steel, the lid of steel and windowlite. Th
bottom of the tank slopes fa
ward to allow débris to fall in
the gully. This is separated fro
the tank by a perforated tra
one for each separate compa
ment. These trays have hand
which allow them to be taken o
separately for cleaning purpos
The gully is connected to t
drainage system by way of
wide-bore tap. The size of ea
compartment is 2 ft. × 2 ft
the water-level is 2–3 in.

Suitable electric heaters
100 W. capacity can be obtain
from the General Electric Co
pany. Combined with therm
stats as supplied by the Brit
Thermostat Company, Sunbu
on-Thames, these heaters c
easily keep the temperature
the required level and the tan
need no attention apart fr
feeding and changing the wate

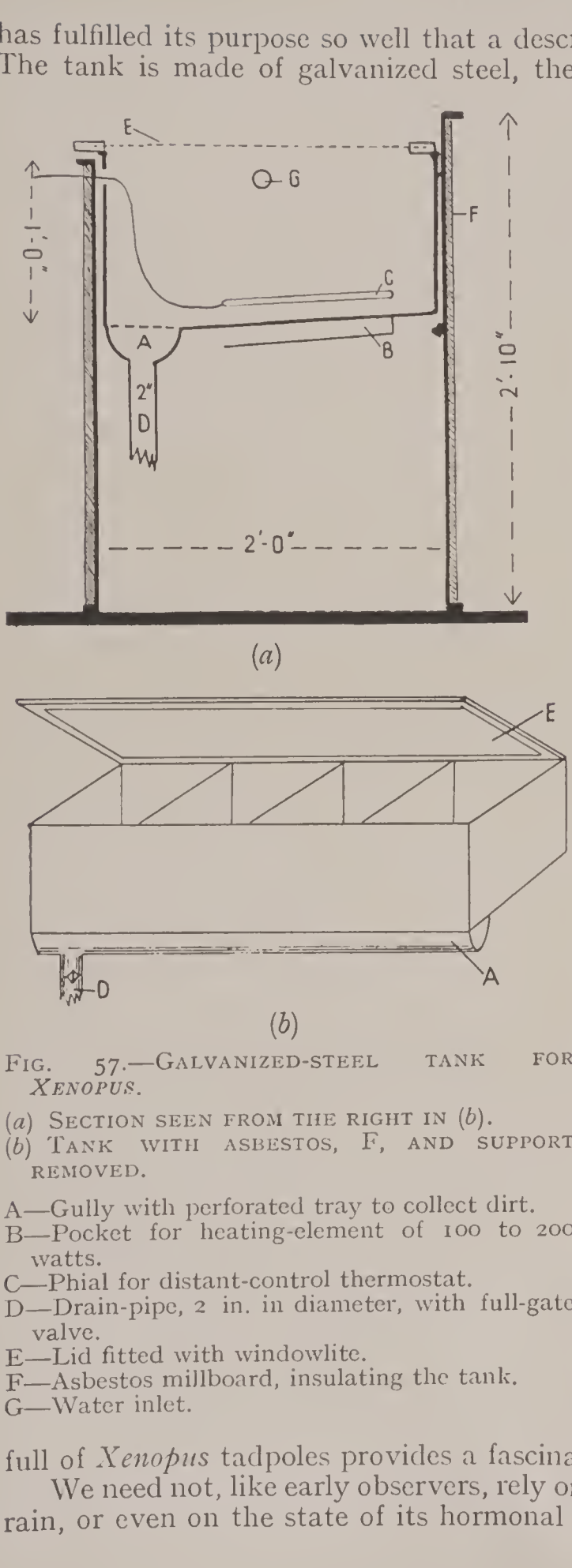

FIG. 57.—GALVANIZED-STEEL TANK FOR *XENOPUS*.

(*a*) SECTION SEEN FROM THE RIGHT IN (*b*).
(*b*) TANK WITH ASBESTOS, F, AND SUPPORT REMOVED.

A—Gully with perforated tray to collect dirt.
B—Pocket for heating-element of 100 to 200 watts.
C—Phial for distant-control thermostat.
D—Drain-pipe, 2 in. in diameter, with full-gate valve.
E—Lid fitted with windowlite.
F—Asbestos millboard, insulating the tank.
G—Water inlet.

4. Breeding

Xenopus does not and cann
in freedom, live in Great Brita
and the breeding of sufficie
supplies has not been found
easy task. Our imports co
from the swamps of South Afric
the animal's life-cycle and
velopment are closely adapted
the climatic and biological chan
there, and these are not eas
imitated in the laborato
Under ideal conditions, prefera
including the use of a hot-ho
kept at 24° to 26° C., it is, ho
ever, quite possible to br
Xenopus over here, and
accompanying illustration (F
58 *b*) may indicate that a ta
full of *Xenopus* tadpoles provides a fascinating spectacle indeed.

We need not, like early observers, rely on the animal's good-will, on artifi
rain, or even on the state of its hormonal cycle. We can influence this cy

v injecting gonadotrophic preparations into the female. If, after that, it is ıt into a separate tank with a few well-sized males, which need not be injected, will be found in copula with one of them (Fig. 56), within ten minutes. The her males are then removed from the tank. This should be stocked with a ood bunch of well-growing water plants. Canadian water weed or *Myrio-yllum* is suitable, and the pair will soon be seen swimming about, the female taching eggs one by one to the plants, the male making small croaking noises ıile fertilizing the eggs. Hundreds of these will be found attached to the ants after a night. If the temperature is suitable the microscope shows that ese eggs are in the first stages of division, and to avoid disturbance the parents e now removed from the breeding-tank.

The tadpoles hatch within a few days and attach themselves to the plants means of a cement gland situated in the place of the future mouth (Fig. *a*). The task of the breeder is easy enough at this stage. His trouble ırts when the tadpoles have used up their yolk lipoids, formed a mouth, rned upside down, and start balancing themselves head downwards in the ter, gulping it up at regular intervals and filtering it after the manner of ales. The most beautifully turbid water, stocked with an abundance of glena, volvox, algæ and infusoria, is soon cleared, but the tadpoles remain venous. They will not, like our indigenous species, swim towards a piece of ten wood to nibble away the infusorial growth on it. All they will do is to nd in the water and gulp. To feed them, an emulsion must accordingly be ovided. In peace-time, liver ground up in a mortar was found extremely table. There is no danger in making the water of a tank containing, say, e hundred tadpoles, completely turbid by pouring in emulsion. The tad-es clear it within a few hours. Dried-egg powder, which has now become re available, would probably do equally well. Under ideal conditions poles grow up in from 3 to 4 months (Fig. 59). They lose their tail, undergo tamorphosis and emerge as tiny " froglets," not easy to feed since from now they need more solid particles than emulsion. At this stage *Daphnia* or *lops* are almost indispensable. Only after 2 or 3 years will these froglets of a size suitable for laboratory work. Hence the reluctance of *Xenopus* kers to embark on large-scale breeding operations.

Supplies. Specimens used to be obtained from Peers' Zoo and Snake Park, lerley St., Cape Town, and now come from the South African Inland Fisheries artment, Stellenbosh near Cape Town. The latter firm also supplies suit-containers (" carboys ") with ample room for the toads to swim about on long journey. A consignment of 500 toads can be delivered from house to se for about £100. The lapse of time between ordering and receiving a ignment is 3 or 4 months and it is advisable to arrange things so that no ignment arrives here during the winter.

Fresh arrivals should be fed and nursed for from 4 to 8 weeks before being in the laboratory, the sexes being sorted out at the same time. Readers rested in further details will be well advised to consult the bibliography plied.

5. Handling

A few words might here be inserted about the handling of the animals ng injections. An attempt to hold these toads by a leg will soon fail. A towel will do if only a few specimens are to be handled at a time. For

(*a*) *Xenopus* Tadpoles, 1 day old, attached to Water Weeds by an (invisible) Thread emerging from the Cement-Gland.

No mouth or anus is as yet formed. Breathing takes place through minute gills.

(*b*) *Xenopus* Tadpoles 6 to 8 weeks old

Note transparency of bodies, appearance of hind legs and typical posture. A tadpo comes up to the surface every 3 to 4 minutes to breathe.

Fig. 58.

FIG. 59.—*Xenopus lævis* DAUDIN. DEVELOPMENT AND METAMORPHOSIS.

larger numbers only a net is suitable ; the material should be of about 5-mm square mesh. In peace-time a kind of netting called " filet " (then obtainab from Lafayette, Regent Street, London), was found eminently suitable. W are now using netting, as sold to anglers, of about 5 mm. mesh, treated wit linseed oil. This makes them a little stiffer but helps, at the same time, t conserve the fabric. The animal's contours can easily be seen and felt an

FIG. 60.—*XENOPUS* TEST JAR WITH PERFORATED LID, METAL CLAMP AND PLATFORM Eggs fall to the bottom of the jar and are out of the reach of the animal.

[Reproduced by courtesy of the *British Medical Journal*.]

injections made through the net. After having been injected the toads a transferred to the test jars, suitable precautions being taken against escape the way. The slippery animals are difficult to catch once they get out of t hand.

6. Test Jars

As a test jar any jam jar is, as a last resort, suitable, if a reliable lid c be provided. Wide-mouthed fruit-preserving jars with perforated glass li fastened by a metal clamp, were found most satisfactory (Fig. 60). Lab

lating to the particulars of the test can be clipped on with the lid. They ould be written with a dermograph pencil so that the writing does not come obliterated by the hot water. For the time of the test the jars are ansferred to an incubator. A simple square tank holding 2 to 3 in. of water, ermostatically heat-controlled at 24° to 26° C. is all that is required.

The animals are not to be fed on the day before nor during a test, and fear s often been expressed that when they have been kept hungry and then been duced to ovulate they might eat their own spawn. It was for this reason that rly observers introduced the perforated platforms as shown in Fig. 60, rough which the eggs fall to the bottom of the jar out of reach of the animal. cases where a few eggs only are laid this precaution may be wise but the iter has kept *Xenopus* in their test jars for a week with their eggs and no od and has never been able to observe any cannibalism of this kind.

7. Determination of the Sexes

Experiments involving ovarian reactions necessitate careful sorting of the es, and although fully-grown specimens show the difference in the cloacal ions well enough, doubt frequently remains with regard to the smaller cimens (Fig. 61 *a*, *b*). This presumably explains the deplorable admixture males constantly found in our consignments " ex overseas," however urgently state that females only are required here. Our illustrations show plainly t the female of the species is possessed of well defined labia placed round the acal opening, and that these labia are missing in the males. Unfortunately ll specimens devoid of labia have often grown up into females under our y eyes, and transfers from the male to the female tank have consequently e made. Outsize specimens 5 in. in length, mouth to anus, which sometimes n up in our transports, are always females. The largest males that have come er observation never exceeded a length of 3 in. Nor are those giant females on to the laboratory. They do not fit into the test jars, they eat more than y are worth, and are useful for breeding purposes only. But even for eding, smaller specimens are preferable because they are more easily managed the males. Colour or colour pattern is no guide to sex.

8. Diseases

Little is as yet known about the diagnosis and treatment of diseases to ch *Xenopus* occasionally succumbs. Epizootics come and go and we can, et, do little to avoid them. Losses on transport may be due to exposure to or heat with over-feeding and pollution of the water, or to so much agita- of the tanks as to prevent the animals from coming up to breathe, with lting suffocation. A night spent in an unheated railway shed somewhere is country in January not unexpectedly killed off all the toads in a con- ment. Of the parasites to whom *Xenopus* serves as a host the most easily rved is a tapeworm, *Dibothriocephalus xenopi Ortlepp*, pieces of which are frequently extruded with the eggs. There is nothing to show, however, this cestode ever causes the death or serious illness of the host.

luch more dangerous and indeed often fatal to the health of a tank full of *opus* are certain trematodes, or flukes, *Diplostomum xenopus*, which enter pericardial sac of the toad and live there for long periods without, at first,

affecting the health of the host seriously. But then, for a reason yet unknown these toads come to the surface in obvious and sudden distress and die within a few hours. Nigrelli and Coates found the pericardial sac of such animal filled with active motile larvæ of this worm and they conclude that the death

(*a*)

(*b*)

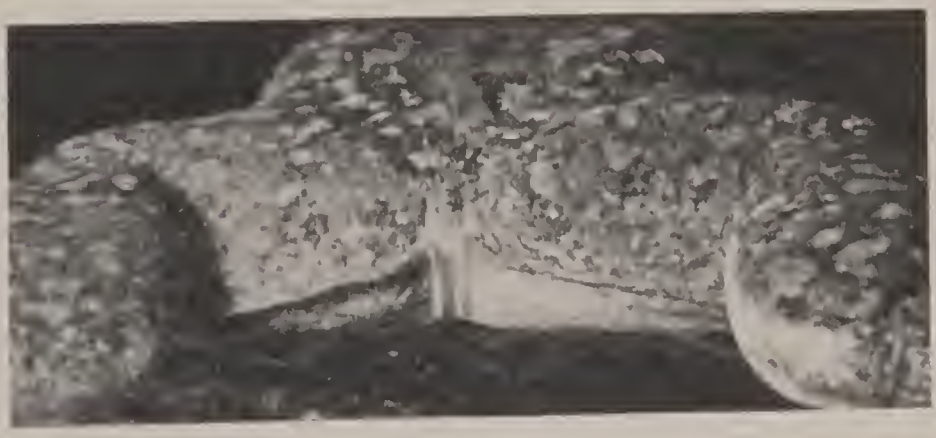

Fig. 61.—Posterior View of: (*a*) Female *Xenopus*, showing Cloacal Valves. (*b*) Male *Xenopus*, showing absence of Valves.

[Reproduced by courtesy of *The Aquarist and Pondkeeper*.]

of the toad is due to cardiac embarrassment. Further experimental work on this infection and any suggestion as to treatment would be welcome.

The one remaining disease of importance, a disease which, like the previous one, can sometimes assume the nature of an epidemic, is still unnamed and unexplained. It is not confined to either sex, to toads that have been used for tests or to imports. I have seen it occur in young "froglets," bred in the

boratory and in large old specimens imported from Cape Town. The leading mptom is a spastic paralysis of the extremities, first of the hind-legs, then, in ore serious cases, of the fore-legs as well. The animal can no longer swim r come to the surface to breathe, and unless speedily transferred to shallow ater it drowns. But if it is kept in a shallow dish at ordinary room-tempera- re the toad often survives. It often refuses food for several weeks but in the d, especially if tempted with a live earthworm, recovers and is then indis- iguishable from its more fortunate brethren. Neither the choice of food, r the temperature, nor a parasite can, so far as we know now, be blamed for is unwelcome disease. It is to be hoped that in the future some investigator ll find the time to clear up its nature.

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Note added in proof to Chapter 20. (*See p.* 250).

Disease control in frogs

Under the above heading S. Meryl Rose (*Science*, 1946, **104**, 330) states that a leprosy like condition is common in laboratory frogs of the four species *Rana catesbiana*, *h clamitans*, *R. palustris* and *R. pipiens*. The initial external signs are minute ulcers on th toes, usually accompanied by the red spots of redleg. Soon the tissues begin to regres the soft more rapidly than the hard, until bare bone protrudes. In extreme cases entir feet are lost, but more often death supervenes before more than parts of toes have dis appeared.

Accompanying the ulceration is a loss of function of afferent but not efferent nerves The disease is said to be readily prevented by keeping the frogs in tap water to whic 0.15 per cent NaCl has been added. Growth is normal and health good in this solutio when the diet is adequate, e.g. meat, bone meal, and salt solution are said to have reduce deaths from all causes almost to zero and it is advocated that all laboratory frogs be kep in the salt solution.—*Ed.*

CHAPTER 22

FRESHWATER FISH

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1. Preferred species. 2. Living-quarters. 3. Aeration of water in the tank. 4. Nutrition. 5. Cleaning. 6. Diseases. 7. Anæsthesia and euthanasia. 8. Sources of supply. 9. Breeding.

1. Preferred Species

It should be stated at the outset that this section deals only with keeping for experimental purposes. It does not deal with fish kept for ornament, as pets, nor with commercial fish-farming, in which various species such as ut, carp and eels are reared under artificial conditions, either for food or to ck natural waters for sporting purposes.

The fish dealt with will be those used for physiological investigations, as t animals in the study of pollution and, to a small extent, for the biological ay of certain substances of medicinal value. The principal species used for se purposes are brown trout (*Salmo trutta* L., sometimes incorrectly known *Salmo fario* L.), rainbow trout (*Salmo gairdneri*, generally though incorrectly ed *S. irideus*), goldfish (*Carassius auratus*), minnows (*Phoxinus phoxinus*) the three-spined stickleback (*Gasterosteus aculeatus*). These species are good experimental subjects, for they live well in captivity and withstand dling. In the writer's experience the so-called coarse fish, perch, roach dace are much more difficult to keep in a healthy condition, and are very sitive to injury in the course of the manipulation necessary in experimental k. Thus whilst it is not denied that the latter can be kept in captivity the owing notes will apply to the former species, for it is with these that most erience has been gained.

2. Living-quarters

For laboratory purposes it is usually convenient to keep fish indoors in ks. The number that can be so accommodated is, however, comparatively ll, and when large numbers or large specimens are required they are best in ponds out of doors.

The form of tank used depends on three factors: the quantity of water lable, the number of fish to be kept and the amount of floor space available. eneral it is desirable to have as large a surface area as possible in relation olume, provided always that there is sufficient depth of water to allow the to swim normally and comfortably. Thus for the same volume of water a 5 ft. long × 2 ft. wide × 1 ft. deep would be better than one half the h and twice the depth. Particularly is this so if the supply of water is ed, for de-oxygenation of the water in warm weather is one of the major

difficulties in keeping fish, and if the water cannot be frequently renewed the exposed surface is a very valuable factor in allowing aeration. A square or rectangular tank is better than a circular one, for the fish can be driven into a corner when it is necessary to catch them. In many laboratories, however, horizontal space is limited, and it is necessary then to use narrow, deeper tanks and to rely on frequent renewal of the water to provide for re-aeration. The writer has found tanks between 4 ft × 3 ft. × 1 ft. 6 in. and 3 ft × 1 ft. × 1 ft very useful, and in fact several tanks of these sizes are, for many reasons, preferable to one large one. They should be installed in a cool place, away from fires and central heating.

Tanks may be made of wood, metal or glass, and of these glass is by far the best. Wood tends to rot and to warp, allowing the water to leak away ; with metal there is a tendency to corrosion which, besides destroying the material of the tank, may cause the water to become poisonous to fish. In both wood and metal (usually galvanized iron) these disadvantages can be overcome to a large extent by the use of preservative paints. Any kind can be used on the outside of the tanks, but for the inside one of the waterproof varieties which have a bituminous basis is essential. Tanks so treated have been used for some years and have proved quite satisfactory. Glass needs no preservative treatment, is very easy to keep clean, and by its transparency allows the stock to be kept under close observation, with advantage to health and economy in feeding. Many experimenters using the smaller species of fish have found it quite satisfactory to keep them in inverted bell-jars or battery jars, which are entirely made of glass ; but when larger tanks are necessary it is essential that the glass should be set in a framework of stout angle-iron and that the bottom should be of slate ; joints between the glass and slate components should be sealed with red lead covered with a coating of bitumen.

Fish love to shelter in dark crannies and nooks, and a considerate aquarist will provide broken flower-pots or other refuges which can readily be removed when the fish have to be caught.

Since it is usually necessary to keep water running through the tanks an outflow system is required which will maintain the water at the desired level. It is a major disadvantage of the small, all-glass tank that this usually has to take the form of a siphon. By using glass tubing of a suitable diameter and fitting the outlet end with a piece of rubber tubing and a screw clip, the outflow can with patience be made to balance the inflow ; but it is a necessary precaution to have the mouth of the siphon inside the tank well below the desired water level, so that small variations, resulting from fluctuations in the rate of supply, do not put the siphon out of action. The disadvantage of the siphon system is that it is very liable to become blocked by pieces of uneaten food or by fæces or even by a fish lying against the mouth, so that the tank overflows. A simple overflow system is, therefore, much to be preferred, for it is then possible to have the waste pipe of such a diameter that it will not choke. In wooden or metal tanks a hole may be bored into the side at the required level, and a pipe fitted to it. This system is simple and foolproof, the only necessary precaution being that the end of the pipe inside the tank should be fitted with a cage of wire netting or of perforated zinc (either of which must be treated with bituminous paint) to prevent small fish from escaping.

The disadvantage here is that when the tank has to be emptied completely the overflow cannot be used. Therefore the most usual system (which indeed

unavoidable in the case of glass tanks) is to bore a hole in the bottom of the nk, whether it be of wood, metal or slate, and fit to it a standpipe which will aw off water at the desired level. Thus when the tank is in normal use the ater is taken off from the surface, but when it has to be emptied the stand pipe n be removed and it can be drained from the bottom. The mouth of the and pipe must, of course, be protected by a cage of perforated zinc, which ay rest in the shape of a cowl on the top of the pipe or preferably form a linder coaxial with the pipe and projecting well above the water surface, to event the escape of fish.

The supply of water to the tanks deserves a little more consideration than generally gets. It is usually derived from a laboratory tap, and to allow the ter to flow straight from the tap to the tank is often satisfactory. The water ould, however, be delivered above, and not below, the water surface, for tap-ter is sometimes deficient in dissolved oxygen, and it is as well to give as ch opportunity for aeration as possible. Consequently it is a desirable nement to allow the water to enter from as great a height and in as fine a as possible. Alternatively the tap may be fitted with some device such as a er pump which permits a current of air to be drawn down with the jet of ter. In that case the mixture of air and water should be discharged at the tom of the tank so that the air bubbles traverse as long a column of water possible. This system is in fact the best for securing adequate aeration and moreover, economical in water.

Particular difficulty would be experienced in maintaining fish in a laboratory ere the water contained small concentrations of heavy metals such as lead. h a difficulty may be overcome by passing the water through absorbent ton-wool or through a Metafilter in which the lead is absorbed.

A more common difficulty arises from the presence of free chlorine in the lic water supply ; chlorine is highly toxic to fish and it must be destroyed ore the water is allowed to enter the aquarium. This is best done by passing water through a column of activated carbon. For a rate of flow of water of gallons per hour, the column should contain about 2 cubic feet of activated on. The material may be contained in two 3-ft. lengths of 9-in. earthen-e pipes joined end to end ; the bottom of the lower pipe may be sealed with expanding pipe-stopper provided with an outlet pipe, and the treated water uld be carried from this outlet through a hose pipe extending up to the level he top of the bed of activated carbon before it is discharged into the arium ; with this arrangement the bed of carbon is kept full of water . 62).

In the laboratory of the Natural History Department at Aberdeen difficulty experienced in keeping minnows alive when they were first introduced to laboratory. The actual cause was not known, but either chlorine or iron was thought to be responsible. The animals dashed round the sink and e incapable of orientating themselves. They tended to swim upside-down. he inflowing water was filtered through earth the toxic effect was removed. apparatus employed as a tank to contain 100 animals was an inverted e-mouthed bell-jar fitted with monel metal gauze and filled with 5 in. of h. This thickness did not retard the waterflow unnecessarily. The earth renewed each month or whenever the animals showed signs of abnormal aviour.

anks containing trout or minnows must be covered, for at times these

fish jump vigorously and unless precautions are taken will jump out on to the floor. A wooden frame made to fit over the top of the tank and covered with wire netting or perforated zinc is all that is necessary, but in large tanks, for ease in handling, it should be made in sections. Goldfish and sticklebacks are not addicted to jumping, and their tanks, if kept indoors, need not be covered. Tanks kept out of doors should always be kept covered, whatever fish they contain, for in rural areas kingfishers and herons will take fish from the most unlikely places, and in towns gulls and even domestic cats are dangers to be avoided.

Generally speaking, laboratory workers will find it possible to keep a sufficient stock of fish in tanks like those described above and, indeed, the keeping of fish in large numbers in ponds is rather beyond the scope of this article. Nevertheless there may be occasions when the necessity for large numbers of fish and facilities for pond-construction exist together, so that a few words on the general principles of the construction and management of ponds may not be out of place.

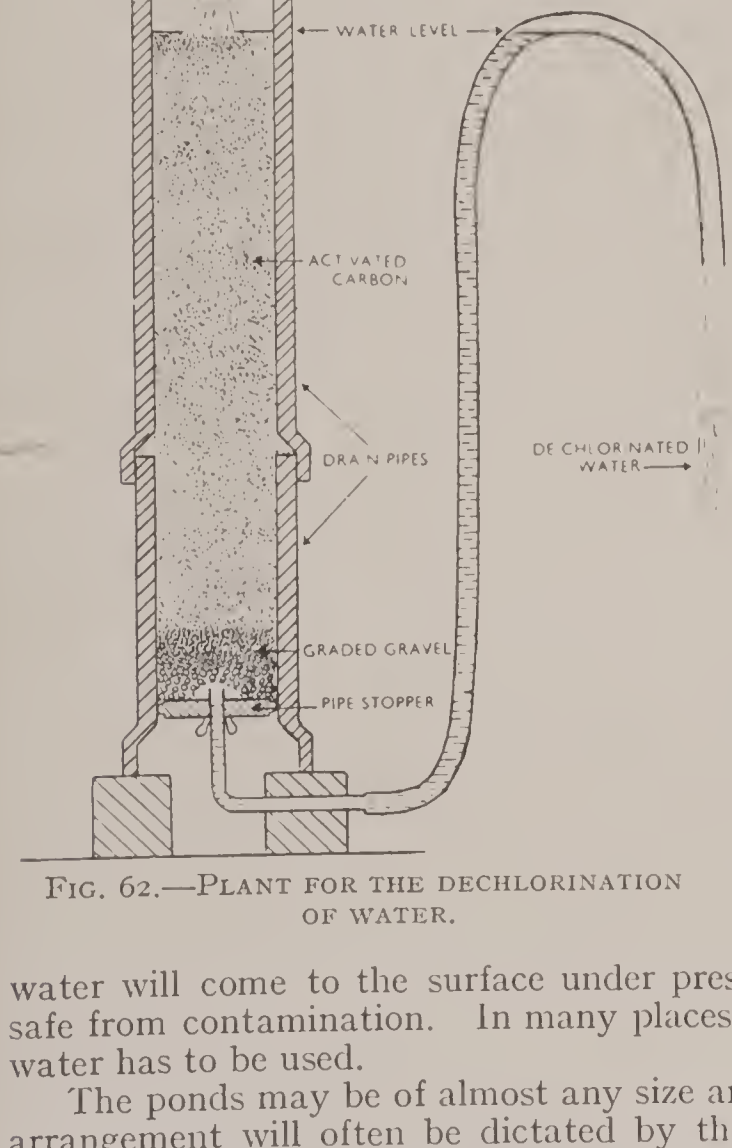

FIG. 62.—PLANT FOR THE DECHLORINATION OF WATER.

An independent water supply is essential, for the quantity of water required is unlikely to be available from the public supply and if it is available is apt to be very expensive. The source must be regular and constant, it must not dry up in hot summers nor flood everything during a wet winter. If the water is obtained from a stream, the stream must be unpolluted and, so far as can be foreseen, not liable to pollution in the future. This is best ensured by obtaining water from a spring or from a borehole in those fortunate areas where underground water will come to the surface under pressure, for springs and boreholes are safe from contamination. In many places they are not available and stream water has to be used.

The ponds may be of almost any size and shape and in fact their form and arrangement will often be dictated by the configuration of the ground, but generally speaking small rectangular ones are preferable. For storing fish, as opposed to rearing them for commercial purposes, the useful size of a pond ranges from 500 to 50 square feet of surface area; above this maximum size there are difficulties in catching fish, and it takes a long time to drain the pond for cleaning; ponds below 50 square feet in area are not worth considering

cause an equal number of fish can be more conveniently kept in tanks. For eping trout a long narrow pond is better than a broad one, for the current, th its effects on aeration and temperature, is felt throughout and there is no ad water. For other species this factor is not so important. It is usual to ke the bottom of a pond slope from the inlet to the outlet so that, for example, e depth at the inlet end is 1 ft. and at the outlet 3 ft. It is sometimes a cessity and generally an advantage to concrete the bottom and sides of the nd; it prevents seepage of the water through porous strata and facilitates aning.

Water may be led into the pond by a pipe or a gutter. In either case a tch or valve is required to control the flow, and to shut it off when necessary; nust also be remembered that fish may escape via the inlet as well as via the let, so that precautions must be taken against this by putting a wire cage er the mouth of the pipe or a grid in the gutter. The outlet is usually a gutter the level required, and this too must be carefully protected by a grid. sides the normal outflow, it is very desirable to fit a drain in the lowest part the bottom so that the pond can be completely emptied. In some ponds s drain is situated in a sump 1 to 2 feet square about a foot below the eral level of the pond bed. This facilitates cleaning and the catching of last few fish that may remain when the pond is drained.

To avoid the depredations of fish-eating birds small ponds can be covered h wire netting fixed to light wooden frames. A wire screen over a pond urally impairs visibility but this can be to a large extent overcome by paint- the wire green. Large ponds cannot be protected in this way, and in them methods of avoiding loss are those of the gamekeeper rather than of the oratory technician.

The number of fish that a given volume of water will accommodate is a ter upon which it is impossible to lay down any hard-and-fast rule, for it ends on the temperature of the water, the rate at which the water is renewed, the length of time during which the fish are to be stored. As a rough guide old aquarists' standard of " one inch of fish (excluding the tail) per gallon of er " is by no means a bad one, and if the water supply is only moderate, if water-temperature is liable to approach the air-temperature in the summer, if the fish are required to be stored for a long period, it may well be adopted. s a 100-gallon tank would be satisfactory accommodation for fifty yearling t 2 to 3 in. long overall, and a pond containing 100 cu. ft. of water would 300 yearlings or 150 two-year-olds.

This is, however, a very conservative standard, and can be considerably eeded for short periods if there is a well-aerated water supply. It has been d, for instance, that 200 yearling trout of an average length of 3 in., or minnows, can be kept in a tank measuring 4 ft. × 3 ft. × 1 ft. 6 in. for e time without difficulty. The danger in a crowd of fish, as in any other rd, is the rapid spread of epidemic disease and, *mutatis mutandis*, the rules ood husbandry apply to fish as they do to rabbits, guinea-pigs and rats.

3. Aeration of Water in the Tank

t has been tacitly assumed in the foregoing that tanks or ponds in which are kept will have a constant stream of water passing through them. e are, however, circumstances where water is either not available or too

expensive for a continuous supply, and in these circumstances the water in th tanks must be aerated. The goldfish-keeper achieves this objective by makin a balanced aquarium in which plants grow and by photosynthesis provide th oxygen for the respiration of the fish. That method is not available to th experimentalist, for he requires that the fish should readily be caught, and tank encumbered with earth and weeds is far from desirable.

There are several ways in which aeration can be accomplished ; they includ the bubbling of air from an electrically-driven blower and the passing of wate pumped from the fish tank back to the tank through a series of filter pump If the activated carbon in a dechlorinating plant is totally enclosed, wate from the plant can be made to pass through a filter pump on its way to th fish tank ; this method of aeration is wasteful in that it necessitates a high rat of flow of water.

It would seem that the simplest method of aeration would consist in passin

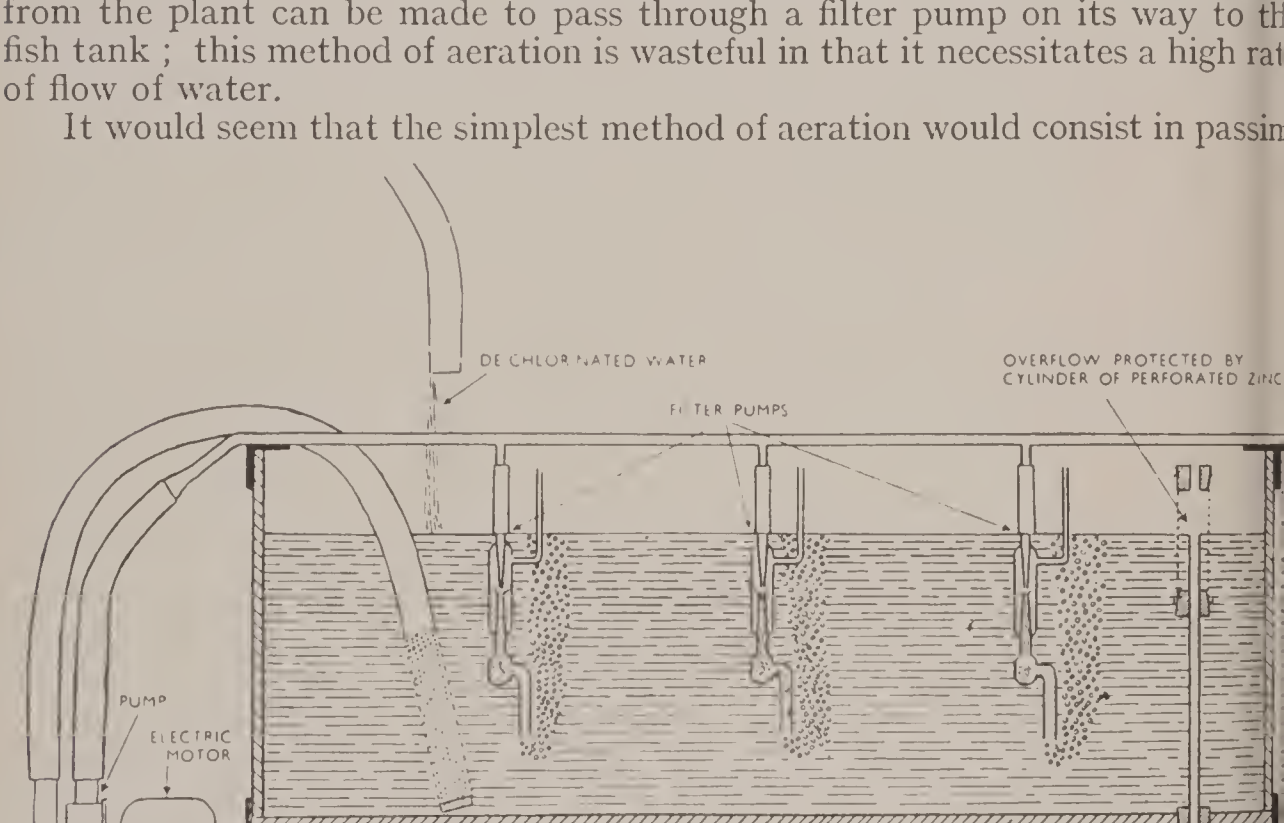

FIG. 63.—CROSS SECTION OF AQUARIUM.

air from an electrically-driven blower through diffuser plates placed at th bottom of the tank. Unfortunately a blower of the size most suitable for th purpose is usually of a type which must be lubricated, and air from it contain a fine spray of oil. If this method is used, therefore, the air must be freed fro oil by passing it successively through an expansion chamber which acts as a oil trap for the grosser particles of oil, through an enclosed vessel filled wit cotton-wool, and finally through a bed of granules of activated carbon. Th stream of purified air should enter the water through a layer of porous materi in order that it may be broken into fine bubbles ; small bubbles have a muc greater surface area than large bubbles per unit volume of air blown, an therefore give up oxygen to the water more easily. Carbon and sintered gla are suitable porous materials for the construction of bubblers ; a bamboo ro into which numerous very small holes have been drilled may be used as a bubbl if proper diffusers are not available.

The method of aeration by which water is continuously pumped from the nk and passed back into the tank through a number of filter pumps does not volve the construction of a purifying train ; the air entrained by the water ıring its passage through the filter pumps does not require purification. centrifugal pump driven by an electric motor of about $\frac{1}{4}$ horse-power is suit-ıle for circulating the water ; such pumps may be obtained fitted with inlet ıd outlet nozzles to which an inch of hosepipe may be secured by means of ıbilee clips. The delivery pipe from the pump is connected through a succes-ın of T-pieces to a number of filter pumps made of glass ; the lower end of ch filter pump should be close to the bottom of the tank, and since the side be of each filter pump may then be under water, air must be drawn from tside the tank through a length of rubber tubing attached to each side tube ig. 63).

Some of the precautions which must be taken in operating an aeration plant this type are immediately obvious. Fish are notoriously fond of nooks and annies ; the open end of the suction hose must, therefore, be protected from h who would investigate its inner darkness. Such investigation would have sastrous results, since the mangled remains of the prying fish would clog the s of the filter pumps and the remainder of the stock might be suffocated. cylinder of perforated zinc should be fastened to the end of the suction hose ; e end of this zinc tube, which should increase the length of the suction hose about 6 or 9 in., should be sealed by flattening and folding its open end. e holes in the perforated zinc should be sufficiently small to prevent uneaten od from being drawn into the pipe.

4. Nutrition

Like other animals in captivity, fish need a regular supply of suitable food keep them healthy. In nature trout and sticklebacks are almost exclusively nivorous and minnows and goldfish omnivorous ; like all other animals y flourish best on a natural diet.

Where a laboratory is situated near an unpolluted stream it may be possible, east during the summer months, to obtain sufficient food such as freshwater imps (*Gammarus pulex*), insect larvæ (particularly of *Diptera* and *Ephe-roptera*) and snails, with the addition, in the case of minnows and goldfish, lgæ and broken-up water weeds, to keep the stock sufficiently well fed with-recourse to artificial foods. Indeed if there are water-cress beds in the nity it may be possible to maintain a natural food supply throughout the r, for they are rich repositories of aquatic fauna.

Even where natural food is not available in sufficient quantity to supply all uirements, it is a very good practice to supplement an artificial diet with it enever possible, for even a small and occasional supply of live food seems to rove the health of captive fish.

In most laboratories, however, reliance will have to be placed on artificial ls, and it is fortunate that in normal times they are readily obtainable rywhere. So far as trout are concerned there is a great deal of information American literature about the composition of suitable diets, chiefly for use ommercial hatcheries where cost is a primary consideration. All kinds of stances have been tried, both separately and in mixtures, from meat, dried k, offals, fish and fish meals to biscuit meal and soya-bean meal. One result

of all this work is the discovery that in spite of their normal carnivorous die trout can utilize a proportion of vegetable protein and carbohydrate.

Very few laboratories, at least in this country, keep such stocks of fish tha the cost of food is a serious problem, and it is usually convenient to feed thei on bullock's heart, liver or ox blood, which can all be readily obtained fro local butchers or slaughterhouses. When more than one day's supply bought at a time the surplus should be stored in a refrigerator, for tainted mea is not good for the fish nor desirable in the laboratory.

The food, minced into particles of a suitable size, for it must be remembere that fish cannot chew, may be given raw or cooked. There is some evidenc that raw food is better than cooked, but raw liver particularly is very messy an wasteful to put through a mincer, and for that reason liver is often boiled. cooked liver is the usual diet, it is a good practice to supplement it with natur food or chopped earthworms, or to give an occasional feed of raw heart.

It is unnecessary to feed the fish oftener than once a day and, particularl in cold weather, every other day may be sufficient. A healthy yearling trou 3 to 4 in. long will consume one or two grams of food a day, but the quantit varies a good deal, apparently according to temperature, for less is eaten whe the water is either very cold or very warm. The amount of food left over fro the previous meal is a good guide in adjusting the rations.

If it is considered that the fish are not eating enough, their appetites ma often be stimulated by adding a lump of salt to the water shortly before feedin time.

Minnows are not very voracious feeders. They will consume bullock- heart in small quantity, but probably the best artificial diet is one composed a mixture of animal matter and a vegetable meal. They have been maintaine for three years at the Water Pollution Research Laboratory at Watford on diet of fish meal obtained from a pet shop. The composition of this meal uncertain, but it obviously contained a proportion of live insect eggs or pupa for insects were observed to emerge if the meal was stored for a period. The meals should be soaked in water before they are fed to the fish.

In the laboratory of the Natural History Department of the University Aberdeen it was found that minnows lived well on a diet of minced liver fed them three times a week. They did even better on live *Daphnia* which we used for the purpose and which solved the food problem when a war-tim scarcity of liver occurred. The breeding medium used for the *Daphn* consisted of horse manure and earth in the proportion of 1 to 4 by weigh with 5 gallons of water to every pound of manure. This mixture was allowe to mature for three days and then the supernatant liquid was transferred the breeding tanks where it was diluted with three or four times its volume water. It was found that 14 gallons of this mixture sustained large quantiti of *Daphnia* for a month without renewal at a temperature of 18° to 19° In most cultures of *Daphnia* a certain percentage of other cladocerans is al present ; if this percentage increases rapidly, it is an indication that the mediu requires renewing. Renewal may be postponed for a few days by adding fi silt and manure enclosed in a bag (after the American fashion of making te which is removed after a day or two ; but this is merely a temporary expedie and the medium eventually has to be replaced. When this is done three qua ters of the contents should be withdrawn from the tank and filtered throug bolting silk to recover the crustaceans. The new medium is added to th

esidue in the tank. On no account should the medium come in contact with ietal.

Goldfish will eat a certain amount of animal food, but they appear to be more ddicted to vegetarianism than most other fish. There are on the market, and otainable from pet shops, numerous proprietary brands of goldfish foods, ome of which seem to be of the nature of crushed dog biscuit, and they are all ore or less satisfactory. A modicum of fresh food is a very desirable addition such a diet and can take the form of chopped earthworms. Goldfish will so eat chopped lettuce. It is said that commercial goldfish farms feed their sh on linseed cake, cattle and horse dung, bread and cooked peas.

5. Cleaning

It is most essential that fish tanks should be kept clean. Uneaten food and ces have a very considerable oxygen-consuming power and most probably their decay produce toxic substances which, in a closed circulatory system, ight easily reach a dangerous concentration. Moreover one of the commonest h parasites, the fungus *Saprolegnia ferax*, is able to live saprophytically, and ganic matter decaying on the bottom of a tank is an ideal pabulum for it.

Tanks are most easily and quickly cleaned by siphoning out a few gallons water through a $\frac{1}{2}$-in. hose pipe ; one end of the pipe is passed to and fro ross the bottom and the debris is removed as with a vacuum cleaner. During is process the end of the tube is held close enough to the bottom to prevent e access of fish. It is, however, a wise additional precaution to discharge e siphoned water into a bucket so that any fish that are entrapped may be overed.

It is possible, though risky, to take advantage of the habit of fish of swimng against the current and to clean a tank by removing the drain plug in the ttom and brushing the debris towards it, but a weak or frightened fish may ily be swept away, and the method is not recommended.

It is also essential to clean carefully the grids protecting the overflow pipe, in a closed circulation the suction pipe, for if these are blocked either the k will overflow, if the water supply is continuous, or the circulation will stop. a tank with a continuous flow, if the bottom is cleaned first the water-level l fall so that the overflow is above the surface and the protecting grid can n be cleaned without any danger of losing fish. In a closed circulation, such that in use in the Water Pollution Research Laboratory, the supply must interrupted and the suction pipe removed, for, unless this is done, it is certain t fish will enter the open end of the pipe whilst the perforated zinc is being aned.

It is an advantage periodically, say once a week or once a fortnight, as ears necessary, to scrub the sides of the tank with a brush. This removes al growths which, besides obscuring the glass, often clog the grids. There is need to remove the fish for this operation, but it is advisable to interrupt flow of water and allow the detritus to settle to the bottom, whence it can siphoned out.

It is a good practice, periodically, say once every three months, to give the ks a thorough disinfection, which helps to keep the fish free from disease. do this, the fish must be removed and accommodated temporarily elsere. If the temporary accommodation is satisfactory the tanks may be

filled with a solution of potassium permanganate or mercurochrome and le to stand overnight. If the tank cannot be spared for so long the sides, botto and particularly the corners should be thoroughly scrubbed with a stron solution of one of these substances, whilst the fittings (grids), overflow c suction pipes, filter pumps, etc., should be steeped in it. It is most essenti that the disinfectant should be well and thoroughly washed out before the fis are replaced, for all disinfectants in quite low concentrations are toxic. Wash ing-out is tedious and often takes longer than the disinfection, but it must b done thoroughly or there is grave danger of losing the stock of fish.

6. Diseases

In this country the subject of fish diseases has received very little attentio On the Continent and in America, where commercial rearing of freshwater fis is a considerable industry, much more study has been devoted to the subje and there are useful handbooks by Hofer (1904), Plehn (1924) and Melle (1927). It must be admitted, however, that further investigation would b very desirable, for diagnosis is by no means certain nor are the remedies alway effective.

Fortunately only a few of the numerous diseases listed in the works me tioned above have so far been recorded from the British Isles, and, whilst th possibility of others occurring must not be overlooked, it would be unprofi able here to do more than mention those which have been commonly known t affect stocks of fish in this country.

The most prevalent complaint of fishes in captivity is fungus disease. affects all kinds of freshwater fish, in natural surroundings as well as in captivit "Fungus disease" is probably an omnibus term covering a variety of ai ments, for neither the symptoms nor prognosis follow the same course in a cases. One variety of it caused considerable mortality amongst salmon Great Britain towards the end of the last century and was investigated b Patterson (1903), who reported that the true killing agent was a bacill (*B. salmonis pestis*), which gained access to the fish's body through a woun and that the fungus (*Saprolegina ferax*) was a saprophyte on the necrotic tissu produced by the bacterial attack. Whether this is the invariable sequence events in fungus disease or whether this or some other fungus does direct attack living tissues of fish has not been determined.

In fishes kept in captivity the disease usually manifests itself by the appea ance on various parts of the body of white cottony tufts, though sometimes thin whitish film is observed before the tufts develop. If neglected the disea will generally spread rapidly to practically all the fish in the tank. Its progre varies. Sometimes the affected fish will die in a day or two with no gro pathological appearances except for some discoloration below the patch fungus, which may be quite small. In other cases the fish will live for week the fungus spreading and apparently eating away the flesh so that, for instanc the caudal region may entirely disappear.

The best treatment is to place the fish in salt water for a period. Sea wate if it is available, is about the right strength and the fish can be left until th show signs of distress, when they should be transferred to fresh water. sea water is not obtainable a solution consisting of 1 oz. of cooking salt to gallon of water may be used. The treatment should be repeated daily f

or 4 days and it is a wise precautionary measure to give all the fish which have een in contact with the disease at least one salt bath during the outbreak. his treatment will cure completely many cases of fungus disease, but some are tractable and seem to respond to no remedial measures.

It has been found in the laboratories of the Natural History Department of e University of Aberdeen that when minnows are affected by fungus or by an identified protozoan parasite it is good policy to weed out all the badly-fected fish and treat the rest with a very dilute solution of mercurochrome for e hour, subsequently adding a small quantity of salt to the stock water. is also stated that the addition of a handful of salt to each 15 gallons of water th sufficient permanganate to colour the water pink, once a week, is a good eventive of disease.

It is also reported in the German literature that fungus can be cured by rubbing the affected areas with a 1 in 1,000 solution of permanganate and en immersing the fish for a few minutes in a 1 in 10,000 solution, but the iter has had no success with this method. In fact in his experience per-anganate solutions are so toxic and have to be used with such care that they e liable to be as dangerous to the fish as the ills they are supposed to cure.

Another common disease is tail rot. This has been very little investigated t appears to be bacterial. The tail fin of the fish becomes bloodshot and a ite streak appears round the edge. Then the soft tissue between the fin ys disintegrates and the rays themselves eventually rot away. The disease, neglected, is soon fatal. The best cure is to hold the fish in a cloth and dip tail in 30-volume solution of hydrogen peroxide.

Fin rot is similar to tail rot, but the unpaired dorsal and ventral fins are ected. It is very difficult to apply hydrogen peroxide effectively to these ans, but some success has been achieved by giving a salt bath as recom-nded for fungus disease. It is stated also that placing the fish for 15 minutes a 1-in-50,000 solution of potassium permanganate is an effective cure.

These are the commonest diseases in this country. If, unfortunately, others uld occur, the reader is advised to consult one of the works mentioned above. nerally speaking, fish disease is not a serious problem in the laboratory, and he water supply is pure and well aerated, the food fresh and plentiful, and tanks not overcrowded and kept clean, little trouble will be experienced.

One difficulty which, though not a disease, may be conveniently mentioned e is cannibalism. Trout will quite commonly eat their brethren if the latter small enough to make a convenient mouthful (and trout have a surprising acity in this respect), and even goldfish will eat goldfish fry. Therefore, of widely different sizes should never be kept in the same tank. A two-r-old trout in a tank of yearlings will eat them at the rate of one a day once gets the taste for them. For the same reason trout and minnows cannot kept in the same tank. Nor is it advisable to keep goldfish with trout, even ugh they be more or less of a size, for the trout will bully the goldfish, ck their scales off, drive them from their food, and generally make life erable for them.

[*Editorial note added in proof.* GUTSELL, J. S. (1946), *Science*, **104**, 85–86, published a preliminary note upon "Sulfa drugs and the treatment of nculosis in trout." He reports encouraging results from the use of sul-merazine mixed with the food at the rate of 5 to 11·5 g. per day per 100 lb. of Sulphathiazole was less effective although it appeared to be of some value.]

7. Anæsthesia and Euthanasia

Anæsthesia. The study of the physiology of fishes is still in a very rud mentary state, and it is doubtful whether in this country any experimen involving more than the simplest operative procedures have been carried ou The commonest uses of fish in experiments necessitating the sanction of viv section licences are their immersion in solutions whose effect it is desired t ascertain, the attachment of marks to various parts of the body for identifica tion purposes in the study of populations and migrations, fin-mutilations fo the same purpose, and simple injection experiments, and in none of these ha it been held necessary that the fish should be anæsthetized. Indeed, for th first group anæsthesia would be impossible, for one of the commonest measur of the effect on fish of substances in solution is the time taken before the fis overturns, i.e. loses its equilibrium and either swims feebly or lies helplessly o its back, when it is presumed to have become unconscious. Consequentl little information is available on the subject of the most suitable anæstheti and this section must, therefore, be incomplete.

Of the substances so far tried, urethane appears to be the best. If fish any species are placed in a dilute solution of, say, 2 or 3 parts per 100,000 the rapidly cease to exhibit signs of consciousness and can be kept quiescent fo considerable periods. When transferred to fresh water they recover rapid and completely.

Dr. J. F. Gaskill reports that soda water is a very good temporary anæsthet with a quick recovery and no harmful effects. The effective agent is dissolve carbon dioxide, which is, however, fatal if the fish are exposed to it for too long

These appear to be the only two substances which have been specifical used as anæsthetics, though doubtless many others exist, and the subje would well repay investigation. It is known, for instance, that fish expose to dilute solutions (1 to 2 parts per 100,000) of phenol, the xylenes and cresc and of cyanides (1 to 10 parts per million), cease to exhibit signs of consciou ness, and when removed to clean water make a rapid and complete recover The disadvantage of these substances is that before this stage is reached th fish become very active and dash about in a frenzied manner, so that they a liable to injure themselves. Yet it may well be that similar substances, not far investigated, might prove to be useful anæsthetics.

Euthanasia. The subject of the best method of killing fish has hardly be investigated, because there is no objective criterion of the sensation of pain these animals. A fish removed from the water will jump about for a time befo it dies ; if put into dilute solutions of phenols or cyanides, it will dash abo before becoming unconscious, and in solutions of chlorine or ammonia of abo 1 part per 100,000 this reaction is even more marked. If it be assumed th abnormal muscular activity is an indication of pain (but it must be remember that it is only assumption) then letting a fish die out of water, or exposing it one of these solutions is not a satisfactory killing method.

Fish are normally killed by a sharp blow on the back of the head. Dea is practically instantaneous, and for most purposes the method is satisfactor It does, however, involve certain physical injury which for some purposes undesirable, and in these cases probably the best method, though no one c confirm that it is entirely painless, is to keep the fish anæsthetized either wi urethane or carbon dioxide until breathing stops.

Another method favoured by humane anglers, and one which is less liable to be bungled than is stunning, is to hold the fish in the left hand, place the forefinger of the right hand on the upper lip of the fish and the thumb on the back of its head ; then bend the head smartly back so as to break the neck.

8. Sources of Supply

Trout, both rainbow and brown, can conveniently be obtained from commercial fish hatcheries, of which there are a number in this country. These will supply fish in any quantity and of almost any size at practically all times of the year, though since both species have very definite breeding seasons it is impossible to obtain the youngest stages except in the winter and spring for brown trout and early summer for rainbow. These firms advertise freely in such papers as the *Fishing Gazette* and *The Field*.

Goldfish, before the war, were largely imported from Italy, and could be obtained from pet shops and even from some of the multiple stores. The larger suppliers were accustomed to advertise in *The Aquarist*. Since the war the import trade has ceased, and it is now very difficult to obtain these fish.

It is not usually possible to buy minnows and sticklebacks. They must be obtained from local streams either by the worker or his laboratory staff, or, better still, if it can be secured, by the assistance of the bait-catcher to the local angling club.

9. Breeding

Trout and minnows will not usually breed naturally in captivity, but goldfish and sticklebacks will.

If goldfish are kept in a pond they will normally spawn in the spring or early summer, but it is essential that there should be submerged weeds, or bundles of sticks specially placed in the pond, to which they can attach their eggs. Very large numbers of fry will be produced, but unless precautions are taken the vast majority of them will be eaten by their parents. Therefore it is necessary to separate that part of the pond where the eggs are laid by putting across a barrier of wire netting of such mesh that the adult fish will not get through. Some of the fry will inevitably stray beyond the nursery and be devoured, but a considerable number will remain and grow until, by the end of the summer, they may be allowed to mix with the older fish. Sticklebacks, like goldfish, will breed in small tanks. They need some aquatic vegetation in which to construct their nests, and if left to themselves will hatch out a considerable number of young. The disadvantage is the pugnacity of the males during the breeding season, for if more than one pair is kept in the same tank (few laboratories can afford one tank to each pair of fish) some mortality is inevitable at this time and may be of such an extent that it is unprofitable to attempt to breed the species.

Although trout will not breed naturally in ponds or tanks, they can very easily be hatched artificially. By pressure along the sides of the abdomen the ova and milt can very easily be expressed from ripe females and males respectively. The eggs are best collected in a dry bowl and the milt then added ; the mixture is well stirred (traditionally with the tail of the cock fish) and water sufficient to cover the eggs to a depth of two or three inches poured in. The bowl is left standing for an hour or so and the eggs are washed and

transferred to grills of glass rods, or to perforated trays of earthenware or per forated zinc (well covered with bituminous paint) which are placed in shallo troughs in a constant gentle flow of water. The purpose of the grills or trays i to allow water to circulate all round the eggs. They must be left thus undis turbed except for the daily removal of dead eggs, which are easily recognizabl by their opacity, until the eyes of the embryo can be seen. Up to this stag mechanical shock is fatal, but thereafter they can be washed, sorted or move wherever they are required. For this reason it is very desirable that the wate used should be free from silt, for an accumulation of silt is fatal to the eggs, y it must not be removed until they are eyed.

The young fish will hatch in from thirty to fifty days after fertilization, th time taken being inversely proportional to the temperature of the water. Fo about a month afterwards they will live without food, sustaining themselv with the yolk-sac, but thereafter they require very careful attention for a fe weeks, for it is at this stage that a heavy mortality is liable to occur. It is b no means difficult to hatch 95 per cent. of the eggs laid down, but to rear 10 p cent. of the resulting alevins to the fingerling stage is no mean achievemen It is, in fact, the hazard of this stage that makes trout-rearing as a method maintaining stocks unsatisfactory for the laboratory worker. It is generall cheaper, and much less trouble, to obtain material from fish-farms than produce it in the laboratory. To those who wish to experiment in this dire tion reference to Coston, Pentelow & Butcher (1936), is recommended.

ACKNOWLEDGEMENTS.

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The writer must also express his thanks to the Ministry of Agriculture and Fisheries, whose service his own experience of keeping fish in captivity was gained, for permission publish this material.

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[*Editorial Note.*—The following additional information was received in response to t questionnaire: Parsons, C. W. (1935), Breeding in captivity of the South Americ Lung-fish (*Lepidosiren paradoxa*). *Nature, Lond.*, **136**, 954; a private communicati from Dr. Margaret Jepps, M.A., D.Sc., Department of Zoology, University of Glasgo indicating that tropical fish are successfully maintained there; and the publicati *Living Specimens in the School Laboratory*, details of which are given in Chapter 23, c taining notes on several species of fresh-water and marine fishes. For marine fish, also Young, J. Z. (1935, *J. exp. Biol.*, **12**, 259). Sudarikov's paper on the change water in tanks for aquatic animals is referred to in Chapter 21, p. 250.]

CHAPTER 23

SOME NOTES ON SPECIES NOT DEALT WITH IN DETAIL IN THE HANDBOOK

1. Introduction. 2. Anthropoids. 3. Dogs and cats. 4. Horses. 5. Other Ungulates. 6. Shrews. 7. Vampire bats. 8. Poultry. 9. Reptiles. 10. Marine Animals. 11. Other invertebrates.

1. Introduction

It is felt that no useful purpose would be served by attempting to deal in a ort work of this kind with some of the more specialized types of laboratory mal. The laboratory maintenance of the monkey, horse, dog and cat is hly specialized and should not be attempted unless the worker concerned s received adequate first-hand information from those who have successfully ried it out. In the notes which follow, reference is made to certain workers o have volunteered to provide the necessary information.

For similar reasons invertebrates have not been included in the main text the book. The few notes which are given below represent information eived in answer to the questionnaire and are included as references.

2. Anthropoids

Prof. S. ZUCKERMAN, F.R.S., of the Department of Anatomy, University of Birmingham, is willing to provide information upon the keeping monkeys under laboratory conditions. Miss E. M. M. HUME, M.A., Division of Nutrition, Lister Institute of Preventive Medicine, London, S.W.1, advise on the nutrition of monkeys. Professor J. F. FULTON, M.D., oratory of Physiology, Yale University School of Medicine, 333, Cedar et New Haven, Connecticut, is preparing a general paper on the care surgical handling of primates based on experiences at Yale during the past en years. (A brief account of the Yale colony was given in 1932 by Fulton n appendix to the monograph *The Sign of Babinski*, published by Charles C. mas, Springfield, Illinois.) A number of papers describing pathological ngs in a series of autopsies on monkeys and chimpanzees has been published by Professor Fulton's colleague, Dr. MARGARET KENNARD, in the *nal of Endocrinology*, and the following two books must be mentioned: RTMAN, C. G. (1934), *The Anatomy of the Rhesus Monkey* (London: ière, Tindall & Cox); Carnegie Inst. (1941), *Embryology of the Rhesus key* (*Macaca Mulatta*).

Among the Soviet material in abstract form in UFAW's possession are rs by BOTCHKAREV, O., & SMIRNOV, A. (1934). [On the question e feeding of anthropoids in captivity], from *The Work of the All-Soviet tute of Experimental Medicine*, Vol. **3**, 191–195, and by SHERVENSKI, V. 2). [The importance of monkey breeding-cages in medicine.] *Ibid.* **4**, 267.

ll anthropoids should be tuberculin-tested on arrival and at fairly frequent vals during confinement.

3. Dogs and Cats

Routine veterinary inspection and advice is strongly recommended, as vaccination against canine distemper and (when the appropriate product available) feline distemper or "feline enteritis." R. F. MONTGOMERI B.Sc., Ph.D., F.R.C.V.S., of the Wellcome Physiological Research Labor tories, Langley Court, Beckenham, Kent, is willing to provide information up the laboratory maintenance of these species. Others who provided inform tion upon dogs in response to the questionnaire included Lt.-Col. A. ANDREASEN, F.R.S.E., I.M.S., O/C Surgical Division, I.M.H. Rawal Pin Punjab, India Command; T. H. B. BEDFORD, M.R.C.P., Department Pharmacology, University of Manchester; Dr. J. N. DAVIDSON, Physiolo Department, Marischal College, Aberdeen; Professor W. H. NEWTO B.A., M.D., D.Sc., University College, London; G. ALEXANDE F.R.C.S., Department of Surgical Neurology, Royal Infirmary, Edinburg and Dr. W. PEREIRA, Harefield Sanatorium and E.M.S. Hospital, Harefie Uxbridge, Middlesex. The last-named was responsible for the design of t warmed, sound-proof kennels for dogs constructed on the roof of the Ro Dental Hospital, Leicester Square, London.

4. Horses

Routine veterinary inspection and advice is again strongly recommend R. F. MONTGOMERIE, B.Sc., Ph.D., F.R.C.V.S., of the Wellco Physiological Research Laboratories, Langley Court, Beckenham, Kent, willing to provide information upon the laboratory maintenance of this spec also.

5. Other Ungulates

Again, routine veterinary inspection and advice are recommended Go are maintained for experimental purposes at Porton Experimental S Wilts., and it is suggested that enquiries be addressed there or to W. BLOUNT, D.Sc., F.R.C.V.S., c/o R.C.V.S., 10, Red Lion Sq., London, W.C

6. Shrews

Pearson (1944) has reported the first successful breeding of shrews captivity. The methods briefly described in his paper are simple and mi presumably be extended to species other th *B. brevicauda*. The essen features appear to be trapping without in y and removal from the traps a as brief a period as possible. No d culty was encountered in maintain such animals alive in glass cages with wood-shavings for litter. The employed was ground or dried commercial dog food to which water and gro meat or ground liver was added.

[PEARSON, O. P. (1944). Reproduction in the Shrew (*Blarina brevica* say). *Amer. J. Anat.*, **75** (1), 39–93. (From the Biological Laborat Harvard University, Cambridge, Mass., our Swarthmore College, Swarthm Penn.)]

7. Vampire Bats

Dr. ARTHUR M. GREENHALL, M.S., Ph.D., Director of the Portl Zoological Park, Portland, Oregon, has worked with this species and will ad

pon its laboratory use. A paper by DITMARS, R. L., & GREENHALL, A. M. (1935), "The Vampire Bat : a Presentation of Undescribed Habits and Review of its History," appeared in *Zoologica*, **29**, 53–76, and was reprinted in 937 in the Smithsonian Report for 1936, pp. 277–296.

8. Poultry

This subject is adequately dealt with in textbooks and bulletins, e.g. *Poultry Husbandry*, by JULL, M.A. (1938), New York : McGraw-Hill Book ompany ; *Diseases of Poultry*, edited by H. E. BIESTER & L. DEVRIES 942), Ames, Iowa : Collegiate Press Inc. ; *Diseases and Parasites of Poultry*, y E. H. BARGER & L. E. CARD (3rd Ed., 1943), London : Henry Kimpton ; ne forthcoming revised edition of the NATIONAL VETERINARY MEDICAL SSOCIATION'S handbook on *Diseases of Poultry* ; *Handbook of Poultry utrition*, by EWING, W. R. (1941), Upper Mnt. Clair ; New Jersey : W. R. wing and various bulletins of the Ministry of Agriculture. According to a rivate communication from T. D. LUCKEY, of the Department of Bio- emistry, College of Agriculture, University of Wisconsin, Madison, the best mi-synthetic ration yet found for both chicks and rats is that given in Table I the paper by BRIGGS, G. M., Jr., LUCKEY, T. D., MILLS, R. C., ELVE- JEM, C. A., & HART, E. B. (1943), "Effect of *p*-aminobenzoic acid when dded to purified chick diets deficient in unknown vitamins" ; *Proc. Soc. p. Biol., N.Y.*, **52**, 7–10.

9. Reptiles

Notes on the feeding and management of **alligators, crocodiles, caimans, vials, turtles, tortoises, terrapins, lizards** and **snakes** are given by E. P. ALKER (1942), in his paper on the "Care of captive animals," in the mithsonian Report for 1941, pp. 305–366.

Walker has written also on "The Care of the Bushmaster and of certain ards in the New York Zoological Park," *Copeia*, No. 1, May 10, 1936. In is, the species of lizard dealt with include *Varanus, Ameiva, Cnemidophorus, nolis* and *Gerrhontus*.

Living Specimens in the School Laboratory (2nd Ed., 1944, Chicago Biological pply House, Inc., Chicago 37, Illinois) provides information upon **alligators, ocodiles, woodland lizards** (*Anolis, Eumeces*), **desert lizards** (*Crotaphytus, eloporus, Heloderma, Ophisaurus, Ctenosaura*), **snakes** (*Thamnopis, Heterodon, trix* and also the Blue racers and other large snakes), **aquatic turtles** (*Pseu- nys, Chrysemys, Amyda, Chelydra*), **terrestrial turtles** (*Terrapene, Gopherus*) d upon terraria. It contains an excellent illustration of the method of force- ding a lizard.

10. Marine Animals

Dr. CURTIS L. NEWCOMBE, Director of the Virginia Fisheries Labora- y of the College of William and Mary and Commission of Fisheries, Williams- rg, Virginia, U.S.A., is willing to provide information concerning *Volsella nissus*, the **ribbed mussel** ; *Littorina irrorata* ; *Urosalpinx cinereus* ; *Mya*

arenaria ; *Venus mercenaria* ; *Alosa sapidisima* ; and *Roccus saxatilis*. Worl on these forms has been, or is being, conducted by members of his group.

Newcombe and his colleagues have overcome at least some of the difficultie experienced in maintaining alive and growing embryonic, larval and postl larval stages of the **common blue crab,** *Callinectes sapidus*, see, e.g., LOCHI HEAD, Margaret S., LOCHHEAD, J. H., & NEWCOMBE, C. L. (1942) *Science*, **95**, 382 ; LOCHHEAD, Margaret S., & NEWCOMBE, C. L. (1942) *Virginia J. Sci.*, **3**, 76–86.

For **cephalopods** see SANDERS, F. K., & YOUNG, J. Z. (1940), *J. Neuro physiol.*, **3**, 501.

In Great Britain supplies of marine animals may be obtained from the Marin Biological Association of the United Kingdom (The Director, The Laboratory, Citadel Hill, Plymouth), and from the laboratory of the Scottish Marine Bio logical Association at Millport. Thanks are due to the late Dr. STANLEY KEMP, F.R.S., for his assistance with this section.

[N.B.—Notes on fish are given editorially at the end of Chapter 22, p. 276.]

11. Other Invertebrates

Leeches. Dr. J. F. GASKILL, M.A., M.D., F.R.C.P., of Uplands, Grea Shelford, Cambridge, notes that the secret of keeping leeches in good conditio is the presence of iron in the water, rusty nails being satisfactory for the pur pose. A paper by him appeared in 1914 in *Philos. Trans.*, B. **205**, 153–211.

Earthworms. Included in the Soviet material in UFAW's possessio is an abstract of a paper by MISENKOVA, E. K., & STEPANOV, M. G (On the method of using the earthworm as an object of pharmacological experi ments), from *The Work of the Stalin Medical Institute*, Jubilee Edn., 194 pp. 108–111, Stalino-Donbas.

Drosophila. Culture methods additional to those given on pp. 437–44 of *Culture Methods for Invertebrate Animals* (New York : Comstock Publishin Co., 1937) include : (i) precautions to avoid loss of laboratory stocks (ii) standardization of culture methods for rearing and handling large number of flies ; (iii) media not requiring agar ; (iv) rearing of *Drosophila* in asepti conditions. All details can be found in the mimeographed publication " Droso phila Information Service," edited by the Department of Genetics, Carnegi Institution, Cold Spring Harbour, N.Y.

* * * * * *

[A paper on the **souslick** or **pouched marmot** as a laboratory animal b D. N. ZASUCHIN (1937), *Laboratory Practice*, **7**, 44–45, is included in th Soviet material in UFAW's possession. It was noted too late to be classi fied.—*Ed.*]

APPENDIX

A CONSPECTUS OF THE ELEMENTS OF STATISTICAL ANALYSIS.

By Major C. W. Hume, M.C., B.Sc., *Chairman of UFAW.*

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TABLE 1. NOTATION

In general use

$)=x_1+x_2+x_3+\ldots\ldots$

nean of $x_1, x_2, x_3 \ldots\ldots$

$/N=\frac{S(x)}{N}$, not $S\left(\frac{x}{N}\right)$

$+b=\frac{1}{a}+b$, not $1/(a+b)$

$+b=b+\sqrt{a}$, not $\sqrt{(a+b)}$

$1\times2\times3\times4\times\ldots\ldots\times n$

≡ denotes an identity, i.e. an equation whose truth follows from the rules of algebra alone.

e, exponential constant 2·71828 . . . . .

P, fiducial probability, § 6.1.

Greek letters, such as σ: parameters in theoretical frequency-distributions (except χ^2, Spearman's ρ and Kendall's τ).

Corresponding roman letters, such as s: estimates of the parameters, calculated from random samples.

Individual to this appendix

', measurements or continuous variates, with sample means $\bar{x}$, $\bar{y}$.

$x-\bar{x}$; $y'=y-\bar{y}$.

hypothetical population means of x, y.

', observed frequencies or discontinuous variates, with sample means $\bar{F}$, $\bar{f}$.

b, expected values of F, f, as calculated from a hypothetical frequency-distribution.

$\chi^2_{[n]}$: the subscript in square brackets denotes the number of degrees of freedom.

he standard normal deviate.

$-x_1+x_2+x_3+\ldots\ldots$

$=(x_1+x_2+x_3\ldots\ldots)^2$

$=x_1^2+x_2^2+x_3^2\ldots\ldots$

he number of observations in a sample.

n, the number of degrees of freedom.

$^{N}S(x)=x_1+x_2+x_3+\ldots\ldots+x_N$ (sum of N observations x).

$\bar{y}_{ab}$, $\bar{y}_a$, $\bar{y}_b$, etc. ; see §§ 7.2, 7.4.

S_1, S_a, S_{ab}, etc. ; see § 7.4.

K_1, K_a, K_{ab}, etc. ; see § 7.82.

k_{xy}, estimated covariance of x and y.

A, B, C . . . . ., names of factors (i.e. independent variates) in an analysis of variance.

(A), (AB), etc. ; see § 7.5.

A, *B*, *C* . . . . . effects of A, B, C . . . . . on a dependent variate.

a, **b**, **c** . . . . phases or levels of A, B, C . . . .

a, *b*, *c* . . . . . observations (or sums of observations) affected by A B, C . . . . .

1. Introduction

The theory of statistics enables an experiment to be planned so that th maximum of information may be obtained from a limited number of observa tions, and conversely it serves to minimize the number of observations require for a given conclusion; it has thus a humane advantage in experiment which impose any degree of hardship on experimental animals. Formerly th biologist's only resource for increasing precision was to use more animals, bu R. A. Fisher and his school have created a new and powerful technique whic has revolutionized the inductive method in this respect.

Scientific research is oriented upon hypotheses of the explanatory typ which account for the facts in terms of real or fictitious mechanisms such a genes, hormones and electrons. The function of such hypotheses is to sugges hypotheses of the descriptive type; that is, quantitative relations whic may hold between observables. Statistical theory yields criteria to indica in terms of probability how far, as a result of an experiment, these quantitati relations are to be regarded as valid; it enables the cogency of an inducti inference to be measured. It enables the whole of the generalizable info mation implicit in a given set of observations to be extracted from ther together with a specification of the degree of reliance to be rested upon it.

The principles underlying the theory must, however, be truly understoo used as a rule of thumb the technique may be misleading. Moreover it canno replace the insight and judgment which enable fruitful hypotheses to l formulated and blind alleys to be avoided.

This appendix assumes a knowledge of the notation used in elementa algebra, which can be learned more easily than a non-mathematical explan tion can be understood.

2. Basic concepts

A *variate* is a quantity, such as the height of a man or the number of cel on a square in a haemacytometer, which can be measured or counted, th measurements or counts varying from instance to instance as a result intentional differences in experimental conditions or of uncontrolled sources experimental error. A variate may be *continuous* (measurable) or *discontinuo* (countable); in the latter case it is usually called a *frequency*. *Observatio* are the numerical values of the measurements or frequencies. A *populati* is the assemblage of all the observations of a given variate that might co ceivably be made. It is a mathematical fiction and is thought of as if it con prised an infinite number of observations of various magnitudes. A *samp* is a random set of observations from which the characteristics of the popul tion are estimated by generalization. (For example, if two sets of simil animals are fed on different diets their initial weights are samples of o population and their final weights are samples of two more populations. Th three populations comprise all the weights that would be obtained if th experiment could be repeated under the same conditions an infinite number times with animals from an infinitely numerous homogeneous stock.)

Statistical parameters, denoted by Greek letters, are constants in a

ıathematical formula which summarizes the values that a variate is likely to ıke ; for instance the mean $\bar{\xi}$ and standard deviation σ of a normal population re parameters in equation (3.32A) below. A *statistic*, denoted by the corre-ɔonding Roman letter, is an *estimate* of a parameter as calculated from a ımple ; for instance the mean $\bar{x}$ and corrected root-of-mean-square deviation calculated from a sample, are estimates of the mean $\bar{\xi}$ and standard deviation of the population from which the sample is taken. While a given parameter ıs one fixed and often unknowable value for the population, the corresponding atistic or estimate has different values in different samples and these values turn may be treated as if they were single observations forming a population their own. The variance (see below) of such a derived population is the *mpling variance* of the statistic in question.

The *mean* of a sample is the average value of the observations in it. Thus e mean $\bar{x}$ of a sample comprising N observations $x_1, x_2, x_3 \ldots x_N$, which e denoted generically by x, is defined thus * :

$$\bar{x} = {}^{N}S(x) \div N$$
$$\text{or } [x]/N$$

The *mean of a population* or of an infinitely large sample, as postulated in distribution formula, § 3, is the *expectation* or *first moment* of the variate ; at is, the sum or integral of all possible values of the variate multiplied each the probability of its occurring. (For example, if I toss four coins and get each time four heads turn up, but nothing otherwise, the expectation of my nnings is £1÷16 or 1*s.* 3*d.* per game, which is the mean in a long series of mes.) The mean differs from the *mode* or most probable value of the variate less the distribution is symmetrical (e.g. in the foregoing asymmetrical case e mode or most probable winning in any game is nil, not 1*s.* 3*d.*, but if I got for every heads the mean and the mode would each be equal to £2 per game four tosses).

A *deviation* † is the difference between any observation and the mean of the ıple to which it belongs. Thus $(x_1 - \bar{x})$ and $(x_2 - \bar{x})$ are two deviations, and *deviate* $(x - \bar{x})$ stands generically for any one of the deviations found in the ıple. Some of these are positive and some negative, and their sum is essarily zero. Thus

$$S(x - \bar{x}) \equiv 0$$

The number of *degrees of freedom* ‡ that are attributed to a statistic is the ıber of observations that are independently used in computing that statistic n a formula. For the mean of N observations there are N degrees of

* In this article " ${}^{N}S$ " denotes a summation containing N terms so that

$${}^{N}S(x) = x_1 + x_2 + x_3 + \ldots + x_N$$

similarly $[x]$ denotes the sum of all the values of x in a sample.

† In this article the deviation corresponding to any observation will be indicated by sh, so that

$$x' = x - \bar{x}$$

‡ In this article it is denoted by a subscript in square brackets, thus :

$$s^2_{[N-1]},\ t_{[n]},\ \chi^2_{[n]}.$$

freedom ; but for estimating the variance (see below) a formula based on devia tions from the mean of the sample is used, and in this case there are (N−1 degrees of freedom because after the values of (N−1) deviations have bee written down the outstanding deviation is not independent ; its value is fixe by them and the mean, which is already in the formula. The number o degrees of freedom applicable to any formula is got by taking the number o observations in the sample and subtracting from it the number of mutuall independent constants that occur in the formula after having been compute from the observations themselves.

The *dispersion* or scatter of the observations constituting a population i measured by the *variance* σ^2, which is the limiting value towards which th *corrected mean square deviation* s^2 tends as the size of the sample is increase The latter is found thus : *

$$s^2 = \frac{{}^{N}S(x-\bar{x})^2}{N-1} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2A)$$

The mean square is *corrected* † by the use as a divisor of the number (N− of degrees of freedom instead of the number N of observations, on the groun that the sample mean $\bar{x}$ is used, not the population mean $\bar{\xi}$. Obviously th procedure gives a true indication when there is only one observation, f equation (2A) then takes the indeterminate form $0 \div 0$ consistently with th fact that a single observation can tell nothing about the dispersion. " *Th sum of squares* " is a slang abbreviation of " the sum of the squares of the devi tions from the mean of a sample," i.e. ${}^{N}S(x-\bar{x})^2$.

The square root σ of the variance σ^2 is the *standard deviation or standa error*. The so-called *probable error* is a number such that there is an even chan of a random deviation being greater or less than it. In a normally distribute population, § 3.32, it is 67·45 per cent of the standard deviation. It commonly used in physics and gunnery but not in biology.

The statistic s^2, equation (2A), is a *consistent estimate* of σ^2, whatever t

* For computation one of the following formulæ should be used :

$$\left.\begin{aligned} {}^{N}S(x-\bar{x})^2 &\equiv {}^{N}S(x-m)^2 - N(m-\bar{x}^2) \\ &\equiv [x^2] - \bar{x}[x] \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (2B)$$

where m is any convenient number and is zero in the second of these formulæ. The latt yield a relative small difference between two relatively large sums ; care must be take therefore, that the difference shall not be swamped by arithmetical errors, as it will be sufficient digits are not retained in the course of the computation.

† *Proof.* Let the mean of the population be $\bar{\xi}$. Obviously

$$(x-\bar{\xi}) \equiv (\bar{x}-\bar{\xi}) + (x-\bar{x}).$$

Let σ^2 be the variance of $(x-\bar{\xi})$; then the variance of $(\bar{x}-\bar{\xi})$ is σ^2/N, by § 5.2. Let be the variance of $(x-\bar{x})$; then, by § 5.1 :

$$\sigma^2 = \sigma^2/N + \zeta^2.$$

Hence

$$\sigma^2 = \frac{N}{N-1}\,\zeta^2.$$

But the best estimate of ζ^2 is the mean square of $(x-\bar{x})$, namely $S(x-\bar{x})^2/N$; her $N/(N-1)$ times this, viz. formula (2A), is the best estimate of σ^2, which is the variance $(x-\bar{\xi})$, and therefore of x since $\bar{\xi}$ is constant.

istribution, in the sense that when averaged over an infinite number of random amples it would yield a mean value equal to σ^2. On the other hand s is not a onsistent estimate of σ; its average value in a normal population, § 3.32, is $/e^{1/4(N-1)}$. However the bias is less than $\frac{1}{2}$ per cent if N is greater than 6.

When measurements are grouped, § 3.1, to facilitate computation, the value alculated for s^2 is as a rule thereby increased. The excess can be approximately corrected by subtracting one-twelfth of the square of the group-width om the computed value of s^2. This (*Sheppard's*) *correction* is valid if the equency tails off to zero as the deviate increases both in the positive and in the egative directions.

The *range* of a sample is the difference between the greatest and least bservations in it. The variance can be estimated from the range (Freeman, able VI), but the sampling variance of this estimate is greater than that of s.

3. Frequency-distributions

3.1 **Groups or classes and their frequencies.** A sample can be divided to groups or classes, each group comprising those observations which ave a particular value or attribute; and the number F of observations in y group is the *frequency* of that group. For instance, if the variate is e number f of cells per square on a haemacytometer, there may be 20 uares forming one group and containing 1 cell each, 43 squares forming a cond group and containing 2 cells each, and so on; the values of the variate haracterizing these two groups are 1 and 2 respectively, and the frequencies for the two groups are 20 and 43 respectively. When the variate is a measurent such as the height of man, instead of being a whole number or frequency, group comprises all the observations whose values lie between specified nits; the *central value* is taken to be the value of the variate for the group, d the *width of the group* is the difference between the two limits. For stance in a batch of recruits there may be one group comprising 3 men with ights between 55 and 56 in., 7 men with heights between 56 and 57 in., and on. Here the group-width is 1 in., the group frequencies F are 3 and 7 pectively, and the values of the variate are taken as $55\frac{1}{2}$ and $56\frac{1}{2}$ respecely. Groups may sometimes be distinguished by qualitative attributes ch as hair-colour instead of quantitative attributes, viz. magnitudes or quencies.

3.2 **Frequency-distributions.**

A formula, graph or table which relates the values of a variate with the rresponding group frequencies is called a *frequency-distribution*. In the case a theoretical frequency-distribution deduced from some hypothesis it is ssible to say what, if the hypothesis is true, is the *probability* p that an servation taken at random shall fall into a given group in a sample containing observations. This probability is defined in relation to the *expected group-quency* ϕ (i.e. the frequency predicted from the theoretical formula) thus:

$$p = \phi/N$$

us it is possible to express a hypothetical frequency-distribution in terms of bability instead of frequency. The probability is a positive fraction; it

cannot exceed 1, which denotes certainty of occurrence, nor be less than 0 which denotes certainty of non-occurrence. If in the case of a continuous variate x the group-width dx is infinitesimally small, so is the probability, and it is then written as dp.

If the distribution of any variate is given as a formula, the mean and variance of the variate can be calculated from the formula. Means and variances in some important distributions are tabulated in Table 4, pp 307, 308

3.3 Distributions of continuous variates (measurements)

3.31 *The standard normal distribution.* A variable quantity is called a *standard normal deviate* if its mean is zero and if the probability dp that a random value shall fall into a group having a central value u and infinitesimal group-width du, § 3.1, obeys the equation

$$dp = \mathrm{U} \,.\, du$$

where

$$\mathrm{U} = e^{-u^2/2} \div \sqrt{(2\pi)}.$$

The probability P that a random value u of a standard normal deviate shall be numerically * greater than some specified value u_0 is found thus :

$$\mathrm{P} = 1 - \int_{-u_0}^{u_0} \mathrm{U} \,.\, du$$

and the probability P′ that a random value u shall be algebraically * less than u_0 is found thus :

$$\mathrm{P}' = \int_{-\infty}^{u_0} \mathrm{U} \,.\, du$$

and u_0, increased by 5 to avoid having to tabulate negative values, is known as the *probit* of P′. It is obvious that

$$\mathrm{P} = 2(1 - \mathrm{P}').$$

The values of U are graphed against u in Fig. 64, and those of P and P′ against u_0 in Fig. 65. Values of P are given against u_0 (there called x) in Fisher and Yates's Table I and in most text-books, and probits are given against 100.P′ in Fisher and Yates's Tables IX, X and XI. The quantity (1 − P) is known as " erf u_0" and has been extensively tabulated. It is convenient to remember that the values of u_0 corresponding to values 0·1, 0·05, 0·01 and 0·001 of P are roughly 1·6, 2, 2·6 and 3·3 respectively.

3.32. *The normal or Gaussian distribution.* If any variate x is so distributed that

$$\frac{x - \bar{\xi}}{\sigma} = u \qquad \ldots\ldots\ldots \quad (3.32\text{A})$$

where u is a standard normal deviate, § 3.31, and $\bar{\xi}$ and σ are constants, then x is said to be distributed normally about a mean $\bar{\xi}$ with a variance σ^2. The distribution is also called Gaussian. The chance that a random value of x

* (−2) is numerically greater but algebraically less than (+1).

shall be numerically greater or algebraically less than a given limit can be inferred from the data for u.

Many variates that are met with in practice have an approximately normal distribution ; for instance heights and weights in homogeneous stocks of men and animals, errors of interpolation in the reading of a scale, and under certain conditions the fall of shot from a gun laid on a given target. Other variates may be convertible into normal variates by a simple algebraic transformation § 4.24 ; for instance by taking logarithms or square roots. The normal dis-

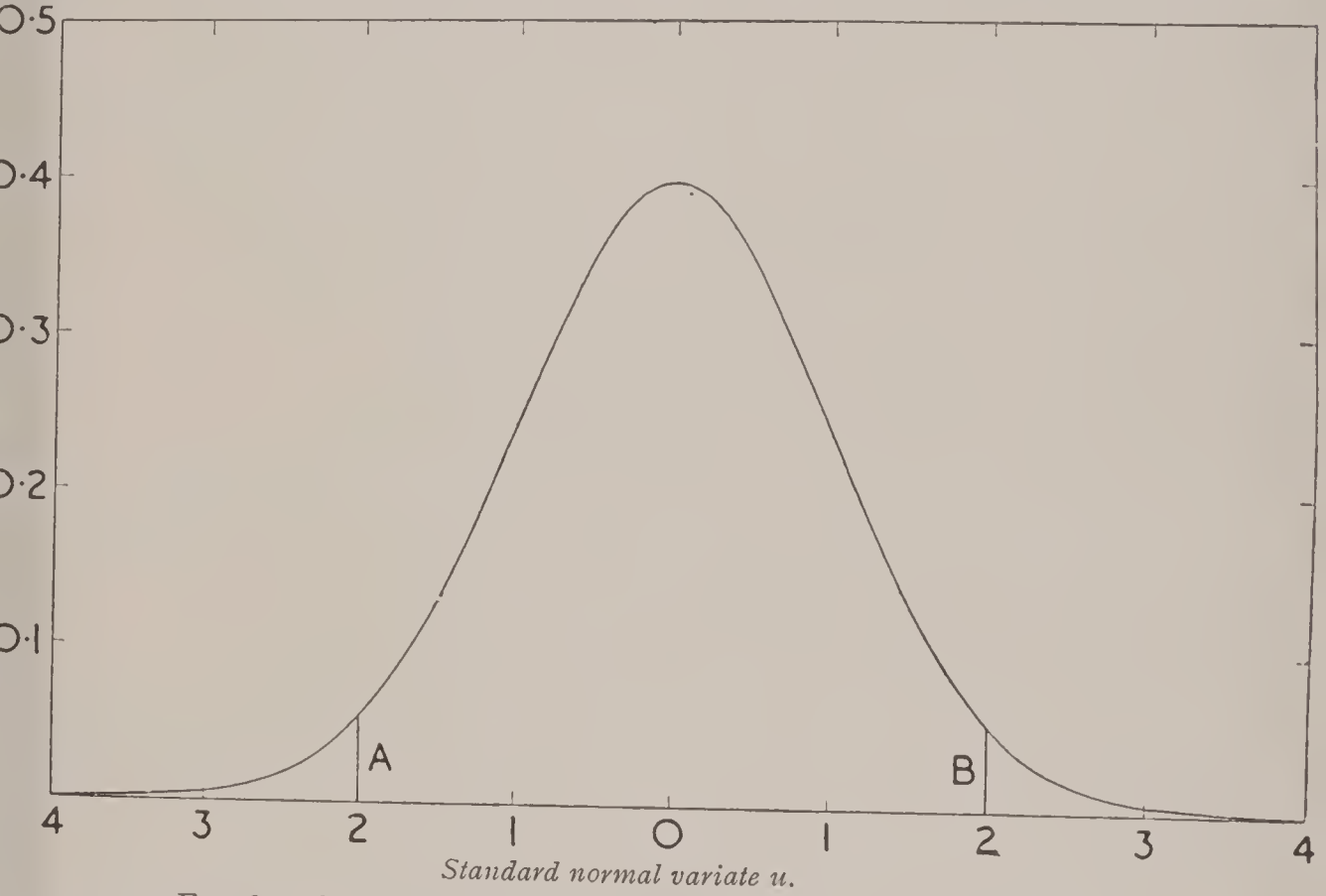

FIG 64.—THE STANDARD NORMAL FREQUENCY FUNCTION U.

U.du is the probability that a random value of a deviate, distributed normally with nit variance, shall fall into a group having a central value u and group-width du. The tal area under the curve is 1 in scale units. The area of the two tails outside A and is 0·045, and is the chance P that u shall be numerically greater than 2. The area to e left of B is 0·977, and is the chance P′ that u shall be algebraically less than (+2).

ibution also affords a first approximation to any distribution in which the bservations cluster about one central mode and become increasingly infre- uent as they deviate more widely from it. The most important property of e normal distribution is, however, that the means of random samples are emselves distributed in an approximately normal way even when the popula- on from which they have been taken is not normal, provided that extreme lues of the variate are not preponderantly frequent ; and as the size of the mples increases the approximation becomes increasingly close. Practical sts of significance are based largely on this fact.

When a normal variate is divided by its standard deviation σ it is converted

into a normal variate with unit variance. It is then said to have been *standardized*.

Certain statistics such as t and z have been devised for the purpose of testing significance. Their frequency-distributions have been deduced for the case in which the samples from which they are to be computed are taken from normally distributed populations, but subject to precautions they may often be applied with sufficient accuracy to samples drawn from populations that are not normal. These statistics are non-dimensional numbers and are not measured in grams, centimetres or other physical units.

3.33 *Student's t as modified by Fisher.* In equation (3.32A) let σ be replaced by an estimate s, equation (2A), computed from a sample comprising N observations x, whereas σ is a constant characteristic of the whole population, as distinct from a sample. Then the distribution is no longer normal, and the normal deviate u is converted into a deviate t whose distribution is graphed in Fig. 66. The number of degrees of freedom appropriate to t is the same as the number appropriate to s^2. Thus

$$t_{[n]}=\frac{x-\bar{\xi}}{s_{[n]}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3.33A)$$

where $\bar{\xi}$ is the mean of the population.

In practice t is generally used for testing not a single observation x but the mean $\bar{x}$ of a sample containing N observations, and since the standard deviation of $\bar{x}$ is estimated to be $s/\sqrt{N}$, § 5.2, we have

$$t_{[n]}=\frac{(\bar{x}-\bar{\xi})\sqrt{N}}{s_{[n]}}$$

When a hypothesis is to be tested by means of t, § 6.22, the population mean $\bar{\xi}$ has to be given by the hypothesis, but the best estimate s^2 of the variance is got by use of the sample mean $\bar{x}$ in the formula ${}^{N}S(x-\bar{x})^2/(N-1)$, so that n is $(N-1)$. If s^2 were calculated from the population mean as ${}^{N}S(x-\bar{\xi})^2$, then n would be equal to N.

The distribution of t, which was worked out by " Student " (W. S. Gosset, Second Brewer to Messrs. Guinness) and modified by Fisher by the use of degrees of freedom n instead of sample-size N, is as follows. If dp is the chance that a value of t, computed with n degrees of freedom from a sample taken at random from a normal population, shall lie between $(t+\frac{1}{2}dt)$ and $(t-\frac{1}{2}dt)$ then

$$dp=\frac{C\,.\,dt}{(1+t^2/n)^{\frac{1}{2}n+\frac{1}{2}}}$$

where C is given approximately * by

$$C=1/e^{1/4n}\sqrt{(2\pi)}$$

* This approximation and those given in §§ 3.34, 3.35 are got by applying Stirling's approximation to gamma functions with fractional indices as well as to factorials, a procedure for which there is empirical justification. The exact value of C is

$$\Gamma(\tfrac{1}{2}n+\tfrac{1}{2})/\sqrt{(2\pi)}\,\Gamma(\tfrac{1}{2}n).$$

The error of the approximation is 2·4 per cent when $n=1$, 0·4 per cent when $n=2$, and increasingly smaller as n increases ; but larger errors obtain in the case of z and χ^2.

When n is large, t degenerates into the standard normal deviate. The chance P that a value of t, calculated from a random sample taken from a normal population, shall be numerically greater than a specified value is graphed in Fig. 66.

3.34 *The mean square contingency* χ^2. A distribution known as that of χ^2 is conformed to by the sum of the squares of N standardized random values of a normal variate whose population mean is known to be zero and whose variance is known to be σ^2. Hence

$$\chi^2_{[n]}=\frac{nS^2_{[n]}}{\sigma^2}$$

the degrees of freedom n being equal to the sample-size N in this case, but not in most of the cases in which χ^2 is used.

The distribution in question is as follows, dp having a meaning similar to that in § 3.33 :

$$dp=\mathrm{D}\,.\,e^{-\frac{1}{2}\chi^2}\,.\,\chi^{n-1}\,.\,d\chi \quad . \quad . \quad . \quad . \quad . \quad (3.34\text{A})$$

where D is, if $n>2$, given approximately by the formula *

$$\mathrm{D}^2=\frac{e^{n-2}}{\pi(n-2)^{n-1}}$$

The chance P that χ^2, calculated from a random sample, shall exceed a specified value is tabulated in most text-books and is graphed in Fig. 67.

When $n=1$, χ is the standard normal deviate u, § 3.31. When $n=2$, $\chi^2=-2\log_e \mathrm{P}$. When $n>30$ we may write approximately

$$\sqrt{(2\chi^2)}-\sqrt{(2n-1)}=u$$

still more approximately

$$\left\{\left(\frac{\chi^2}{n}\right)^{\frac{1}{3}}+\frac{2}{9n}-1\right\}\sqrt{\frac{9n}{2}}=u$$

where u is the standard normal deviate, § 3.31, so that P can in such a case be read from Fig. 65.

If χ^2 lies between two tabulated values ${\chi_1}^2$ and ${\chi_2}^2$, and if P_1, P_2 are the corresponding probabilities read from the table, the precise value of P corresponding to χ^2 can be found by interpolation thus :

$$\log \mathrm{P}=\log \mathrm{P}_1+(\log \mathrm{P}_2-\log \mathrm{P}_1)\,\frac{({\chi_1}^2-\chi^2)}{({\chi_1}^2-{\chi_2}^2)}$$

where $\mathrm{P}_2>\mathrm{P}>\mathrm{P}_1$, so that ${\chi_1}^2>\chi^2>{\chi_2}^2$. Either natural or decimal logarithms may be used.

One function which conforms approximately to this distribution is concerned with observed frequencies f, § 3.1, and with values ϕ predicted for them from some formula. If the formula is correct, so that differences between f and ϕ arise only from chance fluctuations, then, subject to reservations mentioned

* See the footnote, § 3.33. The exact value of D is the reciprocal of $2^{\frac{1}{2}n-1}.\Gamma(\frac{1}{2}n)$.

below, it is approximately true that the following statistic has a χ^2 distribution :

$$S\frac{(f-\phi)^2}{\phi}=\chi_{[n]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3.34B)$$

where n depends on the manner in which ϕ is computed, § 6.31. Neither percentage frequencies nor probabilities may be substituted for f and ϕ, since the numerators in the formula are squared and the denominators are not. Discrepancies due to the fact that χ^2 has been deduced from a continuous distribution, whereas f is discontinuous, are corrected by means of *Yates's correction for continuity*, i.e. by reducing the numerical magnitude of each difference $(f-\phi)$ by 0·5. The extent to which the distribution of χ^2, as calculated from equation (3.34B), approximates to that given by equation (3.34A) then depends, when the discrepancies are due to random errors alone, solely on the values of the expected frequencies ϕ, whatever the parent distribution. No value of any predicted frequency ϕ should be less than 5, and in order that this condition may be satisfied any two or more of the smallest groups may be lumped together to form a single group, the value of n being correspondingly reduced.

3.35 *The variance-ratio* F *and Fisher's z.* If two independent samples taken at random from the same normal population yield estimates s_1^2 and s_2^2 respectively of the variance σ^2, the ratio s_1^2/s_2^2 is the *variance-ratio F*. This is transformed into *Fisher's z* as follows :

$$\begin{aligned} z&=\tfrac{1}{2}\log_e \mathrm{F}\\ &=\tfrac{1}{2}\log_e (s_1^2/s_2^2)\\ &=1{\cdot}1513\log_{10}(s_1^2/s_2^2). \end{aligned}$$

Hence z is positive or negative according as $s_1^2 > \text{or} < s_2^2$. The distribution of z, which is chiefly used in connection with the analysis of variance, § 7, depends on the numbers n_1, n_2 of degrees of freedom with which s_1^2, s_2^2 respectively have been computed. The distribution can be transformed into a symmetrical shape in the following manner :

Write $$v=\frac{n_1}{n_2}e^{2z}=\frac{n_1\mathrm{F}}{n_2}=\frac{[x'_1{}^2]}{[x'_2{}^2]}$$

Then $$dp=\frac{1}{\mathrm{B}(\frac{1}{2}n_1,\ \frac{1}{2}n_2)}\cdot\frac{v^{\frac{1}{2}n_1-1}}{(v+1)^{\frac{1}{2}n_1+\frac{1}{2}n_2}}\,.\,dv$$

where the constant in the denominator is a beta function given approximately when n_1 and n_2 are large, by the formula *

$$\frac{1}{\mathrm{B}(\frac{1}{2}n_1,\ \frac{1}{2}n_2)}=\sqrt{\frac{(n_1+n_2)^{n_1+n_2-1}}{4\pi\,.\,{n_1}^{n_1-1}\,.\,{n_2}^{n_2-1}}}$$

and the integral of the remainder of the expression is the corresponding incomplete beta function.

Thus the distribution of z and those of F and v depend on two different

* See the footnote, § 3.33.

numbers, n_1 and n_2, of degrees of freedom. Let P be the probability that z, as computed from two samples taken at random from the same normal population, shall exceed a specified value z_0; then z_0 can be calculated if P, n_1 and n_2 are given. Values of z_0 are graphed against n_1 and n_2 in Fig. 69 (A) for a value 0·05 of P, and in Fig. 69 (B) for a value 0·01. Fisher and Yates's Table V gives z_0 and F_0 for values 0·2, 0·05, 0·01 and 0·001 of P.

The value of z can be read from the nomogram, Fig. 68, when the values of s_1^2 and s_2^2 have been computed.

When $n_1=1$, e^z becomes Student's t; and when n_2 is infinite, n_1e^{2z} becomes χ^2. When n_1 and n_2 are both large or approximately equal the distribution becomes approximately normal with a variance equal to $\frac{1}{2}(1/n_1+1/n_2)$. When n_1 and n_2 are not too small, z_0 is given approximately by the following formulæ, in which the constants a, b and c have the values given in Table 2 :

$$z_0=\frac{a}{\sqrt{(h-b)}}-ck$$

$$\frac{2}{h}=\frac{1}{n_1}+\frac{1}{n_2}$$

$$k=\frac{1}{n_1}-\frac{1}{n_2}$$

TABLE 2. VALUES OF THE CONSTANTS IN THE APPROXIMATE FORMULA FOR z_0, § 3.35

P	a	b	c
0·2	0·8416	0·6	0·4514
0·05	1·6449	1·0	0·7843
0·01	2·3263	1·4	1·235
0·001	3·0902	2·1	1·925

4 Distributions of discontinuous variates (frequencies)

3.41 *Poisson's distribution* may occur when, as in haemacytometer counting, an intrinsically improbable event (such as the incidence of a cell on a given square) has so many opportunities of happening (there being so many cells) that it does happen on a finite number of occasions. If f is the value of any frequency (the number of cells on any haemacytometer square) and $\bar{\phi}$ is the mean of all the frequencies expected on N occasions (on N different squares), then in Poisson's distribution the number Φ of occasions on which the frequency is expected to have some value f (the number of squares expected to hold f cells each) is $Ne^{-\bar{\phi}}$ times the $(f+1)$th term in the expansion of $e^{\bar{\phi}}$, thus :

$$\Phi=\mathrm{N}\,.\,e^{-\bar{\phi}}\,.\,\frac{\bar{\phi}^f}{f!}$$

By the *expected number* Φ is meant the number calculated on the assumption that the observations conform to Poisson's distribution with a mean $\bar{f}$. The observed number F of such occasions must be a whole number, but the expected number Φ will usually have a fractional part.

3.42 *Binomial distribution.* Let a random sample comprise a group of individuals (such as plants or animals) which can be divided into two sub-groups by reference to a qualitative attribute (such as the presence or absence of some Mendelian character, e.g. colour). Further, let there be some hypothesis which implies that the chance of an individual's falling into one of these sub-groups (e.g. being coloured) is p and that the chance of its falling into the other sub-group (e.g. being albino) is q, where necessarily

$$q=1-p.$$

Then out of any sample comprising f_m individuals let f individuals be observed to fall into the first sub-group. The most probable value of f is pf_m, but in different samples f will have various other values also, f_m being its maximum value. If N samples, each containing f_m individuals, are counted, and if F denotes the number of samples containing f individuals of the first sub-group (coloured) with (f_m-f) of the second (albino), then the value of F is distributed in a super-distribution whose terms form the expansion of the binomial $N(p-q)^{f_m}$. Thus if Φ is the expected value of F

$$\Phi=N\cdot\frac{f_m!}{f!(f_m-f)!}\cdot p^f\cdot q^{(f_m-f)}$$

In short, the individuals forming a sample are classified into two sub-groups according to a qualitative attribute; the samples are classified into groups according to their sub-group frequencies f; and the number F of samples in a given group is predicted to be Φ.

The binomial distribution degenerates into the Poisson distribution when f_m becomes large and p small, pf_m remaining finite; and into the normal distribution when f_m becomes large while p remains finite. *Stirling's approximation for calculating the factorials* is given in § 6.32.

3.43 *Multinomial distributions.* These are based upon sub-distributions in which specimens are classified into, say, k sub-groups distinguished from one another by sets of qualitative attributes such as combinations of Mendelian characters. There will be some hypothesis implying that the chance of a specimen falling into the first sub-group is p_1, into the second p_2, and so on, so that

$$p_1+p_2+p_3+\ldots=1.$$

Let there be N samples containing f_m specimens each and forming a super-sample in which there are Nf_m specimens in all. In any sample let there be f_1 specimens in the first sub-group, f_2 in the second sub-group, and so on, so that

$$f_1+f_2+f_3+\ldots=f_m.$$

Then the samples are distributed in accordance with a superdistribution into groups, each of which is characterized by a different combination of values of

$f_1, f_2, f_3 \ldots$ Let F be the group-frequency, that is to say, the number of amples in which this particular combination of sub-group frequencies occurs, nd let Φ be the expected value of F. Then

$$\Phi = \frac{N \cdot f_m!}{f_1! f_2! f_3! \ldots} p_1^{f_1} \cdot p_2^{f_2} \cdot p_3^{f_3} \ldots$$

4. Conjoined variates

.1 Conjunction, independence, correlation and association

Two variates, x, y, may be *conjoined* with one another in the sense that every ample of one is automatically paired with a corresponding sample of the other .g. men's heights are conjoined with the weights of the same men). If the bservations x are arranged in groups according to their magnitude, then to ch group-value of x there corresponds an *array* of observations y. For stance there may be one group of 12 men whose heights are within $\frac{1}{2}$ in. of) in., so that the group frequency is 12 and the central value x of the group is). Then conjoined with this value of x there will be 12 values of the weight y, hich may be widely scattered. The variate y which is arranged in arrays is lled the *dependent* variate and that x which is arranged in discrete values or oups is called the *independent* variate.

In an entirely different sense of the word, the conjoined variates x and y e said to be *independent* of one another if all arrays of y have the same mean, thin the limits of error. If, however, the means of arrays of y depend on e values of x, then x and y are *correlated*. For instance the heights of adults e independent of their ages but correlated with their weights.

The distribution of a pair of conjoined variates is described as *bivariate*.

When qualitative attributes tend to occur together (as pink eyes tend to conjoined with white fur) they are said to be *associated*. The word " correion " is preferably reserved for measurable qualities.

Regression

4.21 *Linear regression.* When the variates have normal, Poisson, binomial certain other distributions, there may be a *linear regression of y on x*. This a formula for predicting the value of y (or of the mean of an array of y, ich comes to the same thing) that is most likely to be found in conjunction h a specified value of x. Such a formula is derived from a sample as ows, Y denoting the predicted value of y, and $\bar{x}$, $\bar{y}$ the grand means for the ole sample, while b_{yx} is a constant :

$$Y = \bar{y} + b_{yx}(x - \bar{x}) \qquad \ldots\ldots \quad (4.21A)$$

ilarly for a linear regression of x on y :

$$X = \bar{x} + b_{xy}(y - \bar{y})$$

he deviate y' is proportional to the deviate x', as when x is the density and ie pressure of a perfect gas at a fixed temperature, then b_{yx} is equal to

$1/b_{xy}$, but usually this is not the case. The estimated *linear regression coefficients* b_{yx} and b_{xy} are found as follows from N pairs of observations :

$$\left.\begin{aligned} b_{yx} &= \frac{[x'y']}{[x'^2]} = \frac{k_{xy}}{s^2_x} \\ b_{xy} &= \frac{[x'y']}{[y'^2]} = \frac{k_{xy}}{s^2_y} \end{aligned}\right\} \quad \ldots\ldots \quad (4.21\text{B})$$

where s^2_x, s^2_y are estimates of the variances of x and y respectively and k_{xy} is an estimate of the *covariance of x and y* found thus* from N pairs of observations

$$k_{xy} = \frac{[x'y']}{N-1}$$

with $(N-1)$ degrees of freedom.

The population values of b_{xy} and b_{yx} are denoted by β_{xy} and β_{yx}.

It has been assumed that the variance is the same in each array. When this is not true the values of the dependent variate in equations (4.21) must be either loaded in inverse proportion to their variances, § 5.5, or transformed suitably, § 4.24.

The formulæ for the regression coefficients have been deduced from the *rule of least squares*, which requires that ${}^{N}S(Y-\bar{y})^2$ shall be a minimum for the regression of y on x, and vice versa. The regression lines differ from the best fitting straight line, used in physics. in that in determining the latter neither variate is grouped, and the rule of least squares is applied to the distances of the points (x, y) from the line in question.

4.22 *Multiple or planar regression.* Let y depend on two independent variates, w, x. Then if there is a planar regression of y on w and x the most probable value Y of y is found thus :

$$Y-\bar{y} = b_{yw}(w-\bar{w}) + b_{yx}(x-\bar{x}).$$

If w is not correlated with x then b_{yw} and b_{yx} are found as in § 4.21, but otherwise the covariances k_{yw}, k_{xw} of y with w and of w with x must be found by analogy with k_{xy}. Then

$$\begin{aligned} b_{yx} \,.\, s^2_x + b_{yw} \,.\, k_{xw} &= k_{xy} \\ b_{yx} \,.\, k_{xw} + b_{yw} \,.\, s^2_y &= k_{wy} \end{aligned}$$

and b_{yx}, b_{yw} can be computed from these simultaneous equations.

4.23 *Non-linear regression.* While the constants b_{yx} and b_{xy} can always be calculated, equation (4.21B), the distributions of x and y may be such that the resulting equation (4.21A) does not fit the observations. In this case one or both of the variates should be suitably transformed, §4.24, or alternatively a curvilinear regression in ascending powers of the independent variate may be formed, thus :

$$Y = a + bx + cx^2 + dx^3 + \ldots$$

* For computing covariance it is convenient to make use of one of the following formulæ :

$${}^{N}S(x-\bar{x})(y-\bar{y}) = {}^{N}S\{x(y-\bar{y})\} = {}^{N}S\{y(x-\bar{x})\}$$
$$= {}^{N}S(xy) - N \,.\, \bar{x}\bar{y} = [xy] - \bar{x}[y] = [xy] - \bar{y}[x].$$

o as many terms as may be necessary. Rules for calculating the coefficients , b, c, d . . . are given in the text-books ; see, for instance, Tippett, § 9.3 ; ïsher, *S.M.R.W.*, §§ 27, 28 ; and Fisher and Yates's Table XXIII.

4.24 *Transformation of variates : probits.* If there is not a linear regression f y on x or vice versa, there may nevertheless be a linear regression of some ınction of y on x or on some function of x, and it may be possible to guess suitable type of transformation from a graph of y against x. For instance new variate w might be written for $1/y$, or $\sqrt{(y+y_0)}$, or $\log(y+y_0)$, or e^y. gain, if $w=\log y$ a linear regression of w on x will often be obtained if y is a ose of a drug (mg.) and x measures the response. If the standard error of y proportional to y, the standard error of $\log y$ is constant.

The probit transformation is used for expressing a fraction or a percentage terms of the normal standard deviate, § 3.31. Let some all-or-nothing sponse (such as the development of œstrus in response to a specified dose of ı œstrogenic hormone) occur f times out of f_m trials: then f/f_m is called the *antal response.* If we write

$$\frac{f}{f_m}=\frac{1}{\sqrt{(2\pi)}}\int_{-\infty}^{u_0} e^{-\frac{1}{2}u^2}.du$$

ere will be a definite value of u_0 corresponding to each value of f/f_m. If $_m<\frac{1}{2}$, then u_0 will be negative ; for convenience in tabulation, therefore, is added to ensure a positive sign, and (u_0+5) is tabulated as the *probit* of $_m$. (There is often a linear regression of this probit on the logarithm of the se.) Probits are shown graphically in Fig. 65, and it will be noted that f/f_m s been equated to P′, § 3.31.

3 Correlation

4.31 *Interclass correlation.* When linear regressions exist, and in a rough ay in many other cases, the interclass correlation coefficient ρ_1 of the popula- on, which is the limiting value of $\sqrt{(b_{yx}\,.\,b_{xy})}$ for infinitely large samples, easures the relative extent to which the variations of x and y depend on one other as compared with the extent to which they depend on other factors.*

* The nature of regression and correlation can be understood from the relation between pressure p and density d in a perfect gas, in which

$$p=\mathrm{RT}\,.\,d$$

ere T is the absolute temperature and R is a constant. If numerous pairs of values of nd d are measured at numerous temperatures uniformly scattered between T_1 and T_2, n

$$\beta_{pd}=\tfrac{1}{2}\mathrm{R}(\mathrm{T}_1+\mathrm{T}_2)$$
$$\beta_{dp}=(\log \mathrm{T}_2-\log \mathrm{T}_1)/\mathrm{R}(\mathrm{T}_2-\mathrm{T}_1).$$

ow the width $(\mathrm{T}_2-\mathrm{T}_1)$ of the temperature-range is varied while its mid value $\frac{1}{2}(\mathrm{T}_1+\mathrm{T}_2)$ ept constant, then β_{pd} remains constant but β_{dp} grows larger as the width of the range arrowed. Thus the correlation coefficient $\sqrt{(\beta_{pd}\,.\,\beta_{dp})}$ is a small fraction when the perature-range is wide but becomes a large fraction when that is narrow, and is equal +1 when the temperature is held constant so that

$$\mathrm{T}_1=\mathrm{T}_2$$

consequently

$$\beta_{pd}=\mathrm{RT}=1/\beta_{dp}.$$

nce the correlation coefficient between p and d is +1 when T is constant. That ween p and the specific volume $1/d$ is negative.

It is positive or negative according as the larger values of x are conjoine with the larger or the smaller values of y. The correlation coefficient is positiv when x measures a cause that contributes to an effect measured by y (as whe y is a reaction of a person subjected to doses x of a narcotic) ; or when x and measure different effects which are contributed to by the same cause (as when denotes the heights and y the weights of men) ; or when observations hav been selected according to some arbitrary rule which makes large values of conjoin themselves with large values of y (as when x and y both denote quan tities which increase from year to year, or sometimes when samples are selecte by a biased observer). The coefficient is o when x and y are independent, an cannot exceed ± 1 for the closest possible correlation. An estimate r_1 of ρ is found thus :

$$\begin{aligned} r_1 &= \sqrt{(b_{xy} \,.\, b_{yx})} \\ &= \frac{[(x-\bar{x})(y-\bar{y})]}{\sqrt{\{[(x-\bar{x})^2].[(y-\bar{y})^2]\}}} \\ &= k_{xy}/s_x \,.\, s_y \qquad \ldots\ldots\ldots\ldots \quad (4.31\text{A}) \end{aligned}$$

If r_1 is calculated from a series of samples drawn from the same populatio its value varies from sample to sample in accordance with a frequency-distribu tion which is very skew unless r_1 is small and unless, in addition, the sampl are large. A transform z_1 devised by Fisher has, however, an approximatel normal distribution (see Table 4). It is found thus :

$$\begin{aligned} z_1 &= \tanh^{-1} r_1 \text{ (interclass)} \\ &= \tfrac{1}{2} \log_e \frac{1+r_1}{1-r_1} \\ &= 1{\cdot}1513 \log_{10} \frac{1+r_1}{1-r_1} \end{aligned}$$

and is usually called " z " but must be distinguished from the z which stan for $\log_e (s_1/s_2)$, § 3·35.

4.32 *Intraclass correlation.* If the variates x and y are of the same typ (if, for instance, they are the heights of twins) the correlation is called *intraclas* In this case higher precision can be gained by the use of formulæ with mo degrees of freedom and based on the fact that x and y have the same variance thus for N pairs

$$\bar{x} = \bar{y} = {}^{\text{N}}\text{S}(x+y)/2\text{N} = m, \text{ say}$$

$$s^2{}_x = s^2{}_y = \frac{{}^{\text{N}}\text{S}\{(x-m)^2+(y-m)^2\}}{2\text{N}-1} = s^2, \text{ say.}$$

If r_2 denotes the intraclass correlation coefficient :

$$\begin{aligned} r_2 &= k_{xy}/s^2 \\ &= \frac{[x'y']}{[x'^2+y'^2]} \cdot \frac{2\text{N}-1}{\text{N}-1} \end{aligned}$$

a transform z_2 is defined thus :

$$z_2 = \tfrac{1}{2}\log_e \frac{1+r_2}{1-r_2} \text{ (intraclass)}$$

$$= 1{\cdot}1513 \log_{10} \frac{1+r_2}{1-r_2} = \tanh^{-1} r_2$$

e distribution of z_2 is approximately normal (Table 4).

4.33 *Partial correlation.* Let there be three conjoined variates w, x and y uch as the heights, chest-measurements and ages of a group of boys, or essures, densities and temperatures of samples of a perfect gas). Let the ree correlation coefficients estimated as in §4.4 be r_{wx}, r_{xy} and r_{yw} respectively, d let $r_{wx\,.\,y}$ be the value (viz. 1 in the case of the gas) that r_{wx} would take if y re held constant (if boys of the same age were selected or the gas-tempera-re were constant) ; it is known as the *partial correlation coefficient.* Then

$$r_{wx.y} = \frac{r_{wx} - r_{xy} \cdot r_{xw}}{\sqrt{\{(1-r^2_{xy})(1-r^2_{yw})}}.$$

4.4 *Rank correlation.* Let N values of each of two conjoined attributes ch as beauty and brains) be numbered in order of merit or magnitude, and any individual be ranked xth in respect of one (such as beauty) and yth in pect of the other (such as brains), so that x is a whole number ranging from o N and so is y.

The quantity computed like r_1 from equation (4.31A), but with the whole mbers x, y, is known as *Spearman's* ρ, which has a jerky distribution for all values of N. A smoother distribution characterizes *Kendall's* τ, which 2S/N(N−1), where *Kendall's* S is found as follows. The values of x are tten down in numerical order as the whole numbers from 1 to N, and below h value of x is written the conjoined value of y. The N values of y can be red into $\frac{1}{2}$N(N−1) different pairs, in some of which the smaller value will ne first while in the rest the larger value will do so. Kendall's S is got by tracting the number of pairs of the latter type from the number of pairs of former type.

Both Spearman's ρ and Kendall's τ are measures of rank correlation, range m +1 to −1 in value, vary about a mean of zero in different samples when relation is absent, and in that case have distributions which are approxi-tely normal when N is large (even when N is as small as 10 in the case of ndall's τ). A correction for continuity can be applied and a more exact ribution can be used when N is small (Kendall §§ 16.17, 16.23).

4.5 Association

Suppose it to be possible to divide a set of N individuals into two classes nd α so that there are F_A individuals in one class and F_α in the other,

$$F_A + F_\alpha = N$$

let a school comprising N children be divided into F_A children who have ped diphtheria and F_α who have caught it). Let the same individuals be

cross-classified into two other classes B and β so that there are F_B in class and F_β in class β, and

$$F_B+F_\beta=N$$

(e.g. in the school let there be F_B children who have been inoculated and

TABLE 3A. 2×2 CONTINGENCY TABLE; OBSERVED FREQUENCIES

—	Class A	Class α	Total
Class B . .	f_{AB}	$f_{\alpha B}$	F_B
Class β . .	$f_{A\beta}$	$f_{\alpha\beta}$	F_β
Total . .	F_A	F_B	N

TABLE 3B. 2×2 CONTINGENCY TABLE; FREQUENCIES EXPECTED IF CLASSIFICATIONS ARE INDEPENDENT

—	Class A	Class α	Total
Class B . .	$\phi_{AB}=\frac{F_A \cdot F_B}{N}$	$\phi_{\alpha B}=\frac{F_\alpha \cdot F_B}{N}$	F_B
Class β . .	$\phi_{A\beta}=\frac{F_A \cdot F_\beta}{N}$	$\phi_{\alpha\beta}=\frac{F_\alpha \cdot F_\beta}{N}$	F_β
Total . .	F_A	F_α	N

who have not.) Then there will be four sub-classes which may convenient be denoted by AB, Aβ, αB, αβ, with sub-class frequencies f_{AB}, $f_{A\beta}$, $f_{\alpha B}$, $f_{\alpha\beta}$, as shown in a 2×2 *contingency table* in Table 3A. If the two classifications a independent (if inoculation has no immunizing effect) the percentage of A class B will be the same as the percentage of A in class β, and so on; hen the expected values of the sub-class frequencies will be as shown in Table 3 If, however, the classifications are not independent, we have

$$f_{AB}-\phi_{AB}=f_{\alpha\beta}-\phi_{\alpha\beta}=d, \text{ say,}$$

so that

$$f_{A\beta}-\phi_{A\beta}=f_{\alpha B}-\phi_{\alpha B}=-d.$$

If d is positive then A is said to be *associated* with B and *dissociated* from these terms as applied to qualitative attributes correspond to positive a negative correlation as applied to measurable variates. The degree of assoc tion may be measured by Yule's *coefficient of association* Q, defined thus:

$$Q=\frac{f_{AB} \cdot f_{\alpha\beta}-f_{A\beta} \cdot f_{\alpha B}}{f_{AB} \cdot f_{\alpha\beta}+f_{A\beta} \cdot f_{\alpha B}}$$

The value of Q is 1 when A is completely associated with B, zero when A is independent of B, and −1 when A is completely dissociated from B.

In a general contingency table any number of cross-classifications may be represented. Let N individuals be classified in three ways (e.g. let N men be classified by nationality, age and occupation). Let F_1 be the frequency of a class in the first classification (e.g. the number of Frenchmen), F_2 that of a class in the second (e.g. the number aged 21), F_3 that of a class in the third (e.g. the number of gardeners), then if the classifications are independent (if nationality, age and occupation are unconnected with one another) the frequency expected in a sub-class belonging to all three classes (French gardeners aged 21) is

$$N \cdot \frac{F_1}{N} \cdot \frac{F_2}{N} \cdot \frac{F_3}{N} \text{ or } \frac{F_1 F_2 F_3}{N^2}.$$

Each F is the total of the frequencies of all the sub-classes belonging to one particular class, i.e. a marginal total in a contingency table.

5. Compounding and variance of statistics

1 The additiveness of variance

Let three conjoined variates w, x and y be such that

$$y = w + x$$

e.g. w may be the height of a man sitting, x that of a horse's stirrups and y that of a mounted man). Then if there are N sets of observations (N men mounted)

$$^{N}S(y-\bar{y})^2 = {}^{N}S(w-\bar{w})^2 + {}^{N}S(x-\bar{x})^2 + 2\,{}^{N}S\{(w-\bar{w})(x-\bar{x})\}$$

Hence, on division throughout by (N−1),

$$s^2_y = s^2_w + s^2_x + 2k_{wx} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.1A)$$

where k_{wx} is the covariance of w and x, § 4.21. If w is independent of x then k is zero and

$$s^2_y = s^2_w + s^2_x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.1B)$$

Let there be several variates x_1, x_2, x_3 . . . belonging to different populations and let their variances be σ^2_1, σ^2_2, σ^2_3 . . . Let y be a composite variate such that

$$y = l_1x_1 + l_2x_2 + l_3x_3 + \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.1C)$$

where l_1, l_2, l_3 . . . are constants ; then it is easy to show as above that the variance σ^2_y of y is given by

$$\sigma^2_y = l_1^2\sigma_1^2 + l_2^2\sigma_2^2 + l_3^2\sigma_3^2 + \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.1D)$$

provided that the x's are not correlated with one another.

It follows that if the deviation $(y-\bar{y})$ is made up of parts δy_1, δy_2, δy_3 . . . attributable to two or more independent factors (as the error in range of a shell is attributable to variations in air-density, laying, propellant, etc.) so that

$$y - \bar{y} = \delta y_1 + \delta y_2 + \delta y_3 + \ . \ . \ .$$

and if the variances of these parts are σ_1^2, σ_2^2, σ_3^2 . . ., then for the variance σ^2_y of y

$$\sigma^2_y=\sigma_1^2+\sigma_2^2+\sigma_3^2+ \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.1E)$$

5.2 Variance of mean of sample

Now suppose that the infinite populations from which the x's are drawn have equal means and variances, or that the x's are drawn from the same population. Then

$$\sigma_1=\sigma_2=\sigma_3= \quad . \quad . \quad . \quad =\sigma_x, \text{ say.}$$

Let there be N of these x's forming a sample, and choose constants l such that

$$l_1=l_2=l_3= \quad . \quad . \quad . \quad =\frac{1}{N}$$

Then, equation (5.1C), $y=(x_1+x_2+x_3+ \quad . \quad .)/N=\bar{x}$

and from equation (5.1D) the variance $\sigma^2_{\bar{x}}$ of the mean $\bar{x}$ is found thus :

$$\sigma^2_{\bar{x}}=\sigma^2_y=N\left(\frac{\sigma^2_x}{N^2}\right)=\frac{\sigma_x^2}{N} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.2A)$$

Thus the variance of the mean of a random sample containing N observation is 1/N times the variance of a single observation.

5.3 Variance of the difference between two observations

In equation (5.1C) write

$$l_1=1 \; ; \; l_2=-1 \; ; \; l_3=l_4= \quad . \quad . \quad . \quad =0,$$

so that y is the difference between two observations, x_1 and x_2. If these observations are drawn from different populations whose variances are σ^2_1 and σ^2 respectively, then

$$\sigma^2_y=\sigma_1^2+\sigma_2^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.3A)$$

If, however, they are drawn from the same population so that σ_1 is the same a σ_2, it follows that the variance of their difference is twice the variance of a sing observation.

5.4 Variance of the difference between two means

Let $\bar{x}_1$ be the mean of a sample containing N_1 observations out of a popul tion with variance σ^2, and $\bar{x}_2$ the mean of a sample containing N_2 observatio out of the same population, so that $\bar{x}_1$ and $\bar{x}_2$ themselves belong to two differe populations having variances σ^2/N_1 and σ^2/N_2 respectively. Then in equatic (5.1C) write

$$l_1=1 \; ; \; l_2=-1 \; ; \; l_3=l_4= \quad . \quad . \quad . \quad =0$$

and in equation (5.3A) write

$$\sigma_1^2=\sigma^2/N_1 \; ; \; \sigma_2^2=\sigma^2/N_2.$$

Thus y is the difference between the two means, and for its variance σ^2_y:

$$\sigma^2_y=\sigma^2\left(\frac{1}{N_1}+\frac{1}{N_2}\right).$$

5.5 **Loaded means**

Let there be l_1 observations, § 3.1, having a value x_1, l_2 having a value x_2, and so on. Then the mean $\bar{x}$ is found thus, if all the observations are equally reliable:

$$\bar{x}=\frac{l_1x_1+l_2x_2+l_3x_3\ \ldots}{l_1+l_2+l_3\ \ldots}$$

Alternatively the constants l may be weights so chosen as to give the greater influence to those observations x which are considered to be the more reliable. For instance, to find the loaded mean of single observations drawn from populations having different variances, the reciprocals of these variances are taken as the constants l.

.6 **Compounding estimates of σ^2**

If two estimates $s^2_{[m]}$, $s^2_{[n]}$, of σ^2 have been deduced from two different samples with m and n degrees of freedom respectively, a loaded compound estimate with $(m+n)$ degrees of freedom and correspondingly higher precision may be obtained thus, the constants l, § 5.5, being the respective numbers of degrees of freedom:

$$s^2_{[m+n]}=\frac{ms^2_{[m]}+ns^2_{[n]}}{m+n}$$

Analogous formulæ may be used for compounding three or more estimates.

7 **Compounding values of χ^2**

Let there be several samples drawn from the same population and let the values of χ^2 as calculated from them be $\chi^2_{[l]}$, $\chi^2_{[m]}$, $\chi^2_{[n]}$ . . . with l, m, n . . . degrees of freedom respectively. Then a compound value of χ^2 can be got by adding these values together and adding together also the degrees of freedom, thus

$$\chi^2_{[l+m+n+\ \ldots]}=\chi^2_{[l]}+\chi^2_{[m]}+\chi^2_{[n]}+\ \ldots$$

8 **Compounding and variance of statistics of conjunction**

5.81 *The correlation coefficient.* Table 4 gives the variances of statistics connected with correlation.

When g values of z_1, § 4.31, have been found from g different samples whose es N may differ, the loaded mean $\bar{z}_1$ can be found as in § 5.5, the multipliers l ng the reciprocals (N−3) of the variances. Thus

$$\bar{z}_1=\frac{{}^g\mathrm{S}\{(\mathrm{N}-3)z_1\}}{{}^g\mathrm{S}(\mathrm{N}-3)}$$

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The respective values of z_1 may be corrected for bias, if necessary, by deduction of $r_1/2(N-1)$ in each case. An average value of r_1 can be deduced from this result, but estimates of r_1 should not be averaged without such preliminary transformation to z_1.

The variance of $\bar{z}_1$ as derived from equation (5.1D) and from the variance $1/(N-3)$, Table 4, is

$$1/{}^{g}S(N-3).$$

The intraclass correlation coefficient r_2 and its transform z_2, §4.32, can be treated similarly, $(N-1{\cdot}5)$ being substituted for $(N-3)$.

5.82 *Linear regression coefficients.* An estimate s^2_b of the variance of the regression coefficient b_{yx} of y on x can be deduced from equations (4.21B), (5.1D) and (7.81B), the variance of y being based for this purpose on deviations from the regression line instead of the grand mean $\bar{y}$. Then

$$s^2_b = \frac{1-r^2_1}{r^2_1} \cdot \frac{b^2_{yx}}{N-2} \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.82A)$$

with $(N-2)$ degrees of freedom.

5.83 *The variance of a prediction from a regression.* The value Y predicted from equation (4.21A) for any one value x will vary when regressions are derived from different samples taken from the same population. Its variance will be compounded of a part $(1-r_1^2)[y'^2]/N(N-2)$, contributed by the sampling variance of the term $\bar{y}$, viz. $1/N$ times the variance of $(y-Y)$ as deduced from § 7.81, and a part, viz. $(x-\bar{x})^2 \cdot s^2_b$, equation (5.1D), contributed by the last term in equation (4.21A). Thus for the estimate s^2_Y of the variance of Y :

$$s^2_Y = \frac{(1-r^2) \cdot {}^{N}S(y-\bar{y})^2}{(N-2)} \cdot \left(\frac{1}{N} + \frac{(x-\bar{x})^2}{{}^{N}S(x-\bar{x})^2}\right)$$

The variance s^2_Y is, therefore, least for the mean value $\bar{x}$ of x, and increases as the values of x and Y diverge from the means of the sample. *A fortiori* the predicted value becomes increasingly unreliable if the regression formula is used for extrapolation.

5.9 Compounding measures of significance

In a test of significance, § 6, a value of u, t, z or χ^2 as computed from a sample is found, from tables, to be associated with a test probability P, § 6.1. When such probabilities P_1, P_2, P_3 . . . P_k have been derived from k different samples drawn from the same population, a composite value P_K can be formed and yields a more sensitive test of significance. Write

$$\chi^2_{[2k]} = -4{\cdot}6052(\log_{10} P_1 + \log_{10} P_2 + \ldots)$$

Then P_K is the probability derived for the value of $\chi^2_{[2k]}$ from the table of χ^2 with $2k$ degrees of freedom. The values of P_1, P_2 . . . must be found by interpolation, § 3.34. The above formula depends on the fact that $-2 \log_e P = \chi^2$ for 2 degrees of freedom.

TABLE 4. MEANS AND VARIANCES IN HYPOTHETICAL DISTRIBUTIONS

VARIATE	DISTRIBUTION	MEAN	VARIANCE
u	Standard normal	0	1
x	Normal or Gaussian	$\bar{\xi}$	σ^2
Mean $\bar{x}$ of N observations x having any distribution.	Usually normal when N is large.	$\bar{\xi}$	$\frac{\sigma^2}{N}$
Estimate s^2 of variance of normal population, from sample of N observations.	$\frac{\sigma^2\chi^2_{[N-1]}}{N-1}$	σ^2	$\frac{2\sigma^4}{N-1}$
Estimate s of standard deviation of normal population, from sample of N observations.	$\frac{\sigma\chi_{[N-1]}}{\sqrt{(N-1)}}$	$\sigma \,.\, e^{-1/4(N-1)}$	$\frac{\sigma^2}{2N}$
f	Poisson	$\bar{\phi}$	$\bar{\phi}$
Estimate s^2 of variance in Poisson population, from sample of N.	Poisson with group-frequency intervals of 1/N.	$\bar{\phi}$	$\frac{\bar{\phi}}{N}$
f	Binomial $(p+q)^{fm}$	pfm	$pqfm$
Mean $\bar{f}$ of sample of N from binomial distribution.	Binomial $(p+q)^{Nf_m}$ with group-frequency intervals of 1/N.	pfm	$\frac{pqfm}{N}$
Fisher's z	§ 3·35	0	$\to\frac{n_1+n_2}{2n_1n_2}$ when $n_1\to n_2$ or $n_1\to\infty$, $n_2\to\infty$.
Student's $t_{[n]}$	e^z when $n_1=1$, $n_2=n$.	0	$\to 1$ when $n\to\infty$
$\chi^2_{[n]}$	ne^{2z} when $n_1=n$, $n_2=\infty$.	n	$2n$
Yule's coefficient of association Q.	—	0	$(1-Q^2)^2\frac{[f-1]}{4}$
Estimate r_1 of interclass correlation coefficient from N pairs of observations.	Very skew unless N is large and ρ small.	ρ_1	$\to\frac{(1-\rho_1{}^2)^2}{N-1}$ when $N\to\infty$
viz. $\tanh^{-1}r_1$ (Fisher's interclass z).	Almost normal	$\tanh^{-1}\rho_1+\frac{\rho_1}{2(N-1)}$	$\frac{1}{N-3}$
viz. $\tanh^{-1}r_2$ for estimate r_2 of intraclass correlation coefficient (Fisher's intraclass z).	Almost normal	$\tanh^{-1}\rho_2-\frac{1}{2}\log_e\frac{N}{N-1}$	$\frac{1}{N-\frac{3}{2}}$

TABLE 4. MEANS AND VARIANCES IN HYPOTHETICAL DISTRIBUTIONS—*contd.*

VARIATE	DISTRIBUTION	MEAN	VARIANCE
Linear regression coefficient b_{yx} of y on x.	—	β_{yx}	$\frac{b^2_{yx} \cdot (1-r_1^2)}{(N-2)r_1^2}$
Y predicted from linear regression	—	$\bar{\eta}+\beta(x-\bar{\xi})$	$\frac{b^2_{yx}(1-r_1^2)}{(N-2)r_1^2} \cdot \left(\frac{1}{N}+\frac{x'^2}{[x'^2]}\right.$
Spearman's ρ when correlation is absent.	Nearly normal when N is very large.	0	$\frac{1}{N-1}$
Kendall's S when correlation is absent.	Nearly normal when $N>9$.	0	$\frac{N(N-1)(2N+5)}{18}$

6. Tests of significance

6.1 Levels of significance

The variation in magnitude of observations may be attributable partly t measured factors (as the growth-rates of rats to measured differences in diet and partly to unreckoned factors (as to unknown differences in the pri history and genetic constitution of the animals). The effects of unreckone factors constitute the *experimental error.*

For the purpose of a statistical test of significance a hypothesis from whic a definite frequency-distribution can be calculated must be devised. It known as a *null hypothesis* because its commonest form is " that the differenc between the observations are attributable to experimental error alone." the null hypothesis is disproved the result of the experiment is usually positive.

A statistic such as u, t, χ^2 or z is computed from the observations, and t *fiducial probability* P corresponding to its experimental value can be taken fro the tables or graphs on the basis of the frequency-distribution implied by t null hypothesis. This P is the probability, if the null hypothesis is true, th a random value of the statistic shall be not less than the experimental valu If there were no experimental error the test function would be 0 on the n hypothesis, and P is the probability that so large a value as that which actually found should occur as a result of experimental error alone.

The null hypothesis is rejected if P lies below some conventional value known as the *level of significance,* which commonly is 0·05. To say th $P=0{\cdot}05$ is to say that the odds are 19 to 1 against the occurrence of such a lar value of the statistic if the null hypothesis is true ; i.e. against its magnitu being due to experimental error alone. If u, t, χ^2 or z is great, then P is sma and the significance of the experimental result is great. The result is significa if P is below the level α of significance.

* An exception occurs, for instance, when the null hypothesis is " that the populat mean is $\bar{\xi}$ " where $\bar{\xi}$ is some numerical value predicted by theory, or when a formula being tested for goodness of fit.

When the object of an experiment is to make an estimate $\bar{y}$ of the mean $\bar{\eta}$ of a population, the result will be expressed thus :

$$\bar{\eta}=\bar{y}\pm\delta\bar{y}$$

where $\delta\bar{y}$ is an error such that there is a chance α of $\bar{\eta}$ lying outside the stated limits, α being the level of significance. For any value α, such as 0·05, of P a value t' can be found from the table of Student's t for the appropriate degrees of freedom. Then

$$\delta\bar{y}=s't'$$

where s' is the estimated standard deviation of $\bar{y}$. In recording such a result the relevant value of α must be stated.

6.2 **Tests of significance for continuous variates (measurements)**

6.21 *Mean and variance both given.* The standard normal deviate, viz. $(x-\bar{\xi})/\sigma$, § 3.31, can be used for testing significance in populations whose mean $\bar{\xi}$ and variance σ^2 are known or postulated, or as an approximation to certain other statistics when samples are large. The deviation $(\bar{x}-\bar{\xi})$ of the mean of a sample comprising N observations has a standard deviation $\sigma/\sqrt{N}$ and

$$u=\frac{\sqrt{N}}{\sigma}(\bar{x}-\bar{\xi}).$$

The null hypothesis is " that u differs from 0 as a result of the random errors measured by σ." The probability P′, as deduced from the null hypothesis, that u should have a value algebraically less than that which it actually has can be read from Fig. 65, and if P or $2(1-P')$, § 3.31, is less than the level of significance the null hypothesis is rejected. Thus if $P'>0{\cdot}975$ so that $P<0{\cdot}05$, the null hypothesis will be rejected on the 5-per-cent level of significance. If then the sample differs from the known population in having received some experimental treatment, a value of u greater than 2 indicates, at the 5-per-cent level of significance, that the treatment has had a significant effect.

6.22. *Mean given, variance estimated.* Student's t, § 3.33, is applicable to samples of a population whose mean is known or postulated while its variance has to be estimated from the sample ; or to the comparison of two variances when one of them has only one degree of freedom. Its chief use is for comparing the means of two samples. If N_y children have been subjected to an experimental treatment (such as a special diet) and there are N_x control children, and if the reactions (such as logarithmic growth-rates) of the experimental and control children are denoted generically by x and y respectively, then the null hypothesis is " that the difference $(\bar{x}-\bar{y})$ between the means is attributable to experimental error." The value of t for the deviate $(\bar{x}-\bar{y})$, whose mean is zero on the null hypothesis, is found with (N_x+N_y-2) degrees of freedom thus

$$t_{[N_x+N_y-2]}=\frac{\bar{x}-\bar{y}}{s_m} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6.22A)$$

where s_m is the estimate of the standard deviation of the difference of the means and is found thus, § 5.6 :

$$s_m = s \times \sqrt{\left(\frac{1}{N_x} + \frac{1}{N_y}\right)}$$

s being the estimate of the standard deviation of the population so that, § 5.1 :

$$s = \sqrt{\frac{[x'^2]+[y'^2]}{N_x+N_y-2}}$$

If, however, the observations are associated in pairs (for instance if the children are pairs of twins) we may find t with $(N-1)$ degrees of freedom for the mean of the N differences d between members of pairs, and d will be distributed about a mean of zero in an approximately normal manner if the null hypothesis is true. Thus :

$$d = x - y$$

$$t_{[N-1]} = [d].\sqrt{\frac{N-1}{N.[d^2]}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (6.22B)$$

The value of t having been found, the fiducial probability P is read from a table or the graph, Fig. 66, and if it is less than the level of significance to which the experimenter is working the null hypothesis is rejected ; the difference between the two means is in that case significant.

6.23 *Mean and variance both estimated.* The variance ratio F and Fisher's z, § 3.35, are applicable when both the mean and the variance of the population have to be estimated from samples. One or the other is usually used in connection with the analysis of variance, § 7, which yields two independent estimates of variance, s_1^2 and s_2^2, and the null hypothesis is that these are estimates of the same variance σ^2. Hence the ratio F will, on the null hypothesis, tend to unity and z will tend to zero. When s_1^2 and s_2^2 have been computed, z can be read from the nomogram, Fig. 68, and the fiducial probability P can then be found from Fig. 69. If z is so large that P lies below the working level of significance, which may be 0·05 as in Fig. 69 (A), then s_1^2 differs significantly from s_2^2.

6.24 *Tests based on several samples.* Let v be any statistic whose distribution is approximately normal with a mean of zero (see Table 4), let σ_v^2 be its variance, and let values of v be computed from g different samples. Then v/σ_v is a standard normal deviate, § 3.31, and therefore, § 3.34 :

$$\chi^2_{[g]} = {}^{g}S\left(\frac{v^2}{\sigma^2_v}\right)$$

This value of χ^2 shows whether the values of v jointly indicate a significant deviation of v from zero. The standard deviation σ_v will vary from sample to sample if the samples are of different sizes.

Whether or not the mean of v is zero, to test whether g independent values

of v differ significantly among themselves a loaded mean $\bar{v}$ is formed, § 5.5, thus :

$$\bar{v} = {}^{g}\mathrm{S}(v/\sigma^2_v) \div {}^{g}\mathrm{S}(1/\sigma^2_v)$$
$$= [v]/g \text{ if the samples are equal in size ;}$$

and for the χ^2 test :

$$\chi^2_{[g-1]} = {}^{g}\mathrm{S}\left(\frac{v-\bar{v}}{\sigma_v}\right)^2$$

The value of σ_v has been assumed to be known independently. It may, however, be deduced from the samples as a loaded estimate, § 5.6, in which case the number of degrees of freedom is reduced by 1.

When g different estimates s^2 of a variance are derived from different samples, which may have different numbers n of degrees of freedom, the following test * will show whether they are consistent within the limits of experimental error or whether the differences between them signify differences between the variances of the populations from which they were drawn. The following functions are calculated :

$$\nu = {}^{g}\mathrm{S}(n)$$

$$\mathrm{B} = \nu \log_e \frac{{}^{g}\mathrm{S}(ns^2)}{\nu} - {}^{g}\mathrm{S}(n \log_e s^2)$$

$$\mathrm{C} = 1 + \left\{ \mathrm{S}\left(\frac{1}{n}\right) - \frac{1}{\nu} \right\} \Big/ 3(g-1) \ .$$

Then $$\chi^2_{[g-1]} = \frac{\mathrm{B}}{\mathrm{C}}$$

A method of compounding fiducial probabilities P derived from different samples and, if necessary, from statistics of different kinds is given in § 5.9.

6.25 *Goodness of fit of a linear regression.* Table 11 and § 7.81 yield (1) the variance of the deviations of means of arrays from the values Y predicted by a linear regression, and (2) the variance of y within arrays. The null hypothesis " that both arise from the same causes " can, therefore, be tested, and if the null hypothesis is not disproved the regression may be taken as valid. For N_x arrays with means $\bar{y}_x$ and numbers N_y of observations per array,

$$z = \tfrac{1}{2} \log_e \frac{(\mathrm{N}-\mathrm{N}_x) \cdot {}^{\mathrm{N}x}\mathrm{S}\{\mathrm{N}_y \cdot (\bar{y}_x - \mathrm{Y})^2\}}{(\mathrm{N}_x - 2) \cdot {}^{\mathrm{N}}\mathrm{S}(y-\bar{y}_x)^2}$$
$$n_1 = \mathrm{N}_x - 2$$
$$n_2 = \mathrm{N} - \mathrm{N}_x.$$

6.26 *Significance of a linear regression.* The variance s_b of the linear regression coefficient b_{yx} of y on x is given in equation (5.82A). Hence for

* *Cf.* Bartlett, M. S., *Proc. roy. Soc.*, **A 160,** 269 (1937).

testing the null hypothesis " that b_{yx} does not differ significantly from zero " or " that there is no significant linear regression " :

$$t_{[N-2]}=b_{yx}/s_b$$
$$=\sqrt{\frac{r_1^2(N-2)}{1-r_1^2}} \quad . \quad . \quad . \quad . \quad . \quad (6.26A)$$

The regression is significant if t is large.

Similarly for testing whether a sample value b differs significantly from some hypothetical value β :

$$t_{[N-2]}=\frac{b-\beta}{b}\cdot\sqrt{\frac{r_1^2(N-2)}{1-r_1^2}}$$

To test whether there is a significant difference between estimates b_1, b_2 derived from two samples whose sizes are N_1 and N_2 respectively, the standard deviation of their difference is estimated as s_d from their respective variances, equation (5.82A), on the basis of § 5.3, and

$$t_{[N_1+N_2-4]}=(b_1-b_2)/s_d$$

6.27 *The significance of a correlation coefficient.* The following tests are applicable when there are linear regressions between the variates ; they may fail, however, if there are non-linear regressions.

When the interclass correlation coefficient r_1 is small (e.g. less than $\frac{1}{2}$) and N is large (e.g. greater than 100) the null hypothesis " that there is no significant correlation " can be tested thus :

$$u=r_1\sqrt{(N-1)}$$

and the null hypothesis is rejected, at the level 0·05 of significance, if $u>2$. A more unbiased test is afforded by equation (6.26A), which tests alike each of the linear regressions and the correlation associated with them.

Interclass linear correlation can also be tested by means of the transform z_1, § 4.31, which for normal populations is distributed almost normally with a variance $1/(N-3)$ about a mean which is zero if there is no correlation, whereas the distribution of r_1 is very skew if r_1 is large or the sample is small. Thus if

$$u=z_1\sqrt{(N-3)}$$

the correlation is significant at the 0·05 level if $u>2$. For testing whether z_1 differs significantly from some hypothetical value ζ_1

$$u=(z_1-\zeta_1)\cdot\sqrt{(N-3)}$$

To find whether there is a significant difference between estimates z_1 and z_1' made from two samples containing N and N′ pairs of observations respectively the standard deviation σ_d of the difference is calculated thus :

$$\sigma_d=\sqrt{\left(\frac{1}{N+3}+\frac{1}{N'+3}\right)}$$

and hence $$u=(z_1-z_1')/\sigma_d$$

Estimates of z_1 derived from several different samples can be dealt with in

accordance with § 5.81 or § 6.24, but if there are many samples the mean $\bar{z}_1$ must be corrected for grouping error and for bias (Fisher, *S.M.R.W.*, §§ 31, 36).

Similar tests are applicable, *mutatis mutandis*, to r_2 and z_2, § 4.32 and Table 4.

A different approach to the problem is afforded by the analysis of covariance, § 7.83 and Table 12.

Presence or absence of a significant degree of rank correlation, § 4.4, can be tested by assuming a normal distribution of Kendall's S, with a standard deviation deduced from the variance, Table 4. A correction for continuity should be applied by subtracting 1 from S if N is small. If Spearman's ρ is used, its less normal distribution can be allowed for by writing

$$t_{[N-2]}=\rho \cdot \sqrt{\frac{N-2}{1-\rho^2}}$$

and using Fig. 66. If N is small, Spearman's ρ can be corrected for continuity in the manner described by Kendall (§§ 16, 17), who gives also exact tests for use when $N<9$.

6.28 *Testing the fit of non-linear regressions.* If a regression departs significantly from linearity, § 6.25, a linear regression may be obtainable by transforming one or both of the variates, § 4.24, and be tested as in § 6.25. Alternatively a curvilinear regression containing a term in x^2 may be tried, 4.23, thus :

$$Y=\bar{y}-bx+cx^2.$$

In this case a z test is carried out with the new values of Y as in § 6.25, except that n_1 has one degree of freedom fewer, so that (N_x-2) will be replaced by $N_x-3)$ in the denominator of z. If the fit is still unsatisfactory, regressions including higher powers of x may be tried successively with the loss of one degree of freedom for each constant $\bar{y}$, b, c, d . . . If the number of constants in the regression is made equal to the number of arrays, the fit can be made perfect ; but any other formula containing the same number of constants can be fitted with equal perfection, so that the regression becomes meaningless.

6.29 *Tests for normality.* The normality of a distribution can be roughly tested by plotting on probability paper, obtainable from Wightman Mountain, Ltd., Artillery House, S.W.1. Of more exact tests the most informative are Fisher's g_1 and g_2. Fisher first defines three constants k in a manner equivalent to the following :

$$k_2=\frac{[x^2]}{N-1}-\frac{[x]^2}{N(N-1)}$$

$$k_3=\frac{NS_3}{(N-1)(N-2)}$$

$$k_4=\frac{N}{(N-1)(N-2)(N-3)}\left\{(N+1)S_4-3\frac{N-1}{N}([x^2]-N\bar{x}^2)^2\right\}$$

where

$$S_3=[x^3]-3\bar{x}[x^2]+2N\bar{x}^3$$

$$S_4=[x^4]-4\bar{x}[x^3]+6\bar{x}^2[x^2]-3N\bar{x}^4$$

then

$$g_1=\sqrt{\frac{{k_3}^2}{{k_2}^3}}\ ;\ g_2=\frac{k_4}{{k_2}^2}$$

Moreover the variances σ_1^2, σ_2^2 of g_1, g_2 are given by :

$$\sigma_1^2 = \frac{6N(N-1)}{(N-2)(N+1)(N+3)}$$

$$\sigma_2^2 = \frac{24N(N-1)}{(N-3)(N-2)(N+3)(N+5)}.$$

The departure of g_1 from zero is an indication of skewness, and g_1 is positive or negative according as the mean is greater or less than the mode. The departure of g_2 from zero indicates non-normal bluntness or sharpness, g_2 being negative if the curve has a flatter top and shorter tails than the normal curve, and positive if the reverse is the case. The significance of these departures from normality can be tested by means of the standard normal deviates g_1/σ_1 and g_2/σ_2.

6.3 Tests of significance for discontinuous variates (frequencies)

6.31 *Goodness of fit.* The square contingency χ^2, § 3.34, is used for testing agreement between frequencies to be expected on the basis of some hypothesis and frequencies observed in an experiment. Let a sample be divisible into k groups of observations, § 3.1 ; these groups may be distinguished from one another by : (1) qualitative attributes, such as Mendelian characters ; or (2) frequencies f in a sub-distribution such as the Poisson series, § 3.41 ; or (3) central values of a continuous variate x which has been grouped to facilitate computation, § 3.1. Let Φ be the expected frequency in any group and F the observed frequency in that group. Then

$$\chi^2_{[n]} = {}^{k}\mathrm{S}\frac{(\mathrm{F}-\Phi)^2}{\Phi}.$$

Yates's correction for continuity, § 3.34, must be applied, and any group in which the expected frequency is less than 5 must be lumped with one or more others to make one larger group, the value of k being reduced to conform. The number n of degrees of freedom is found by the established rule, § . It is $(k-1)$ for a binomial distribution if only the total S(F) is taken from the observations for calculating the values Φ ; but if the probability p also is estimated from the observations, then $n=(k-2)$. For a Poisson distribution both $\mathrm{S}(\Phi)$ and ϕ are usually deduced from the observations as being equal to S(F) and $\bar{f}$ respectively, so that $n=(k-2)$.

Then if the computed value of χ^2 is greater than that corresponding to and the working level of significance, Fig. 67, the null hypothesis " that the observed values agree with the expected values within the limits of error " is rejected.

6.32 *Independence in the 2×2 contingency table.* The variance of Yule's coefficient of association Q, § 4.4, is given in Table 4, and since Q is zero if the classifications are independent a rough test of significance can be made by assuming that the distribution of Q is normal. This assumption is unjustifiable unless the frequencies are large.

In a 2×2 table (Table 3A), the formula for χ becomes

$$\chi_{[1]} = (f_{AB} \cdot f_{\alpha\beta} - f_{A\beta} \cdot f_{\alpha B}) \sqrt{\frac{N}{F_A \cdot F_B \cdot F_\alpha \cdot F_\beta}}$$

and Yates's correction must be applied (by adding $\frac{1}{2}$ to $f_{A\beta}$ and to $f_{\alpha B}$ and subtracting $\frac{1}{2}$ from f_{AB} and from $f_{\alpha\beta}$ if χ is positive, and vice versa); the corrected value is called χ_c. Since there is only 1 degree of freedom, χ_c is the standard normal variate if N is large, and a test of significance can be applied by means of Fig. 65.

Actually the distribution of the above quantity approximates to normality only if the frequencies are large; it becomes skew if any of them falls much below 100, and in that case Fisher and Yates's Table VIII should be used in the manner explained in the introduction to that table.

In lieu of the χ test an *exact test* may be used. The chance p that, when association is absent and the four marginal totals F are specified, the four sub-classes or cells shall have the observed frequencies f is found thus:

$$p = \frac{F_A!\, F_B!\, F_\alpha!\, F_\beta!}{N!\, f_{AB}!\, f_{A\beta}!\, f_{\alpha B}!\, f_{\alpha\beta}!} \quad \ldots\ldots \quad (6.32A)$$

Let f_{min} be the smallest of the observed frequencies f. Then the set of four frequencies f' which satisfies the following two conditions is calculated: (1) the smallest of the four must be $(f_{min}-1)$; and (2) the marginal totals F must be as before. Similar sets with $(f_{min}-2)$, $(f_{min}-3)$ . . . for their smallest frequencies are calculated, and the probabilities p', p'', p''' . . . for all these sets are calculated as in equation (6.32A), except that for the last set 1 is written for 0!. Then the fiducial probability P is found thus:

$$P = p + p' + p'' + p''' \ldots$$

and is the probability that, if the classifications were independent, the observed frequencies would diverge from the expected frequencies not less widely than they do. If $P < 0{\cdot}05$ the divergence is significant at the 5-per-cent level; the classifications are not independent; the attributes on which they are based are associated.

The value of any *factorial, correct to four significant figures*, can be computed from Stirling's approximation thus:

$$\log_{10}(f!) = 0{\cdot}3991 + (f+\tfrac{1}{2})\log_{10} f - 0{\cdot}4343f + 0{\cdot}0362/f.$$

6.33 *Independence in general contingency tables.* In a twofold table, § 4.5, if there be N_1 classes in one classification and N_2 in the other.

Let f be the frequency observed in any sub-class and let F_1, F_2 be the totals for the two classes to which it belongs while $N = S(f) = S(\Phi)$. Then the formula for χ^2, § 6.31, becomes (the square brackets denoting summation as usual)

$$\chi^2_{[n]} = N\left(\left[\frac{f^2}{F_1 \cdot F_2}\right] - 1\right)$$

with n degrees of freedom, where (§ 7.3).

$$n=(N_1-1)(N_2-1).$$

Any sub-class whose frequency is less than 5 must be lumped with another sub-class by adding together the values of f and ϕ and reducing n accordingly. Unless the frequencies are large Yates's correction must be applied, with the result that

$$\chi^2_{[n]}=S\left\{\frac{N}{F_1F_2}(f^2\pm f+\tfrac{1}{4})\right\}-N.$$

the + or − sign being taken according as $f <$ or $> F_1.F_2/N$. If there are three or more cross-classifications, the sub-class frequency is still denoted by f, and the totals for the classes to which the sub-class belongs are $F_1, F_2, F_3 \ldots$, then with Yates's correction

$$\chi^2=S\left\{\frac{1}{F_1}\cdot\frac{N}{F_2}\cdot\frac{N}{F_3}\cdot\ldots\cdot(f^2\pm f+\tfrac{1}{4})\right\}-N.$$

and if χ^2 is large a significant association is inferred.

7. Factorial design and the analysis of variance

7.1 Factorial design

In factorially designed experiments several factors are varied simultaneously instead of all but one being held constant. The observed value of a dependent variate y (e.g. a psychological reaction time) varies in response to the variation of factors or independent variates, some of which (e.g. doses of narcotic or fatigue) may be regulated and measured, while others (e.g. personal constitution) may be regulated or selected but not measured, others (e.g. hours of sleep preceding the tests) may be measured but not regulated or selected, and others again, are neither regulated nor measured but constitute the sources of experimental error. An experiment serves: (1) to test whether the value of y responds in a significant degree to changes in experimental factors; (2) to estimate the magnitudes of such responses as may be found to be significant and (3) to estimate the effect of experimental error.

If a factor is not to be erroneously credited with responses which do not belong to it, it must be free from correlation or association with any other factor; (e.g. if the samples were taken from mixed anti-aircraft batteries composed of young women and middle-aged men, reaction-differences due to age might be erroneously attributed to sex). Correlation (i) between any regulated factor and other regulated factors is prevented by balanced design or equalization, § 7.3; (ii) between it and measured but unregulated factors is eliminated by an analysis of concomitant measurements, § 7.83; and (iii) between it and the error factors is reduced by randomization. *Randomization* consists in so selecting the samples that the error factors have as fair a chance of enhancing the effects attributed to other factors as of opposing them. To avoid unconscious systematic selection, randomization must be effected by drawing lots; for instance by means of random numbers such as those in Fisher and Yates's Table XXXIII.

Factorial design reduces error to a minimum and so reduces the number of observations required for a given degree of precision ; provides a wide basis for inductive inference ; and reveals interactions between factors, § 7.3.

7.2 One factor regulated

7.21 *A randomized block.* Let N observations y or y_{ab} (e.g. psychological reaction times) be arranged in N_a columns and N_b rows, Table 5, so that

$$N = N_a . N_b .$$

Let the observations in any one column be associated with the same phase or level $\mathbf{a}_1$, $\mathbf{a}_2$ or $\mathbf{a}_3$ . . . of some factor A (e.g. with a specified dose of some narcotic), and let $\bar{y}_a$ stand generically for the mean of any column. Let the other factors be randomized (e.g. let the doses be allocated by lot to the persons under test and to all other experimental conditions). For the purpose of the present section the letters **b** are mere labels for the rows, the observations within each column being entered in random order. Such an arrangement is the simplest form of *randomized block.*

TABLE 5. N OBSERVATIONS y OR y_{ab} CLASSIFIED INTO N_a CLASSES **a** AND N_b CLASSES **b**

Phases or levels of B	Phases or levels of A				Totals $[y_b]$	Means $[\bar{y}_b]$
	$\mathbf{a}_1$	$\mathbf{a}_2$	$\mathbf{a}_3$	$\mathbf{a}_4$ . . .		
$\mathbf{b}_1$	y_{11}	y_{21}	y_{31}	y_{41} . . .	$[y_{b_1}]$	$\bar{y}_{b_1}$
$\mathbf{b}_2$	y_{12}	y_{22}	y_{32}	y_{42} . . .	$[y_{b_2}]$	$\bar{y}_{b_2}$
$\mathbf{b}_3$	y_{13}	y_{23}	y_{33}	y_{43} . . .	$[y_{b_3}]$	$\bar{y}_{b_3}$
$\mathbf{b}_4$	y_{14}	y_{24}	y_{34}	y_{44} . . .	$[y_{b_4}]$	$\bar{y}_{b_4}$
. . . . .	. . .	. . .	. . .	. . . .	. . .	. . .
. . . . .	. . .	. . .	. . .	. . . .	. . .	. . .
. . . . .	. . .	. . .	. . .	. . . .	. . .	. . .
Totals $[y_a]$	$[y_{a_1}]$	$[y_{a_2}]$	$[y_{a_3}]$	$[y_{a_4}]$ . . .	$[y]$	$N_b . \bar{y}$
Means $\bar{y}_a$	$\bar{y}_{a_1}$	$\bar{y}_{a_2}$	$\bar{y}_{3a}$	$\bar{y}_{a_4}$	$N_a . \bar{y}$	$\bar{y}$

7.22 *Variances estimated from means of arrays.* Any one of the deviations $y-\eta$) from the mean η of the population may be represented thus :

$$(y-\eta) = A + e$$

where A is a deviation due to the factor A and e is a deviation due to the error actors. Let σ^2 be the variance of y, σ^2_A that of A, and σ^2_e that of e ; that of) is zero. Then since e has been made approximately independent of A by andomization :

$$\sigma^2 = \sigma^2_A + \sigma^2_e \qquad . . . . . \quad (7.22A)$$

by § 5.1. Consider now the variance of the means $\bar{y}_a$ of the columns. To this the error factors contribute a part σ^2_e/N_b, by § 5.3. On the other hand A is constant throughout each column, and its effect on the means of columns is therefore identical with its effect on the observations in each row; i.e. it contributes an amount σ^2_A to the variance of $\bar{y}_a$. Hence

$$\text{the variance of } \bar{y}_a = \sigma^2_A + \sigma^2_e/N_b.$$

A quantity σ^2_a which may be called the *distorted variance between columns* is now defined as N_b times the variance of $\bar{y}_a$, thus:

$$\sigma^2_a = N_b \cdot \sigma_A{}^2 + \sigma^2_e \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.22B)$$

Then σ^2_a is not the variance of y, which is given by equation (7.22A), nor of any other useful variate; nevertheless an estimate s^2_a of it, obtained from a sample, furnishes a sensitive test of significance, § 7.26. Let s^2, s^2_a and s^2_A be estimates, obtained from a sample, of σ^2, σ^2_a and σ^2_A, which latter are the values that would be obtained by taking the averages of s^2, s^2_a and s^2_A over an infinitely large number of samples. Then, consistently with equations (7.22A, B):

$$\left.\begin{aligned} s^2 &= s^2_A + s^2_e. \\ s^2_a &= N_b \cdot s^2_A + s^2_e \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.22C)$$

Now s^2_a, s^2_e can be found as follows, for any sample. An estimate of the variance of $\bar{y}_a$ can be written down from first principles, equation (2A), thus:

$$\frac{s^2_a}{N_b} = \text{corrected mean square of } \bar{y}_a$$
$$= \frac{{}^{N_a}S(\bar{y}_a - \bar{y})^2}{N_a - 1}$$

the degrees of freedom numbering $(N_a - 1)$ since there are N_a data $\bar{y}_a$, and one constant $\bar{y}$ has been calculated from them for use in the formula. Hence:

$$s^2_a = \frac{N_b \cdot {}^{N_a}S(\bar{y}_a - \bar{y})^2}{N_a - 1} \quad . \quad . \quad . \quad . \quad . \quad (7.22D)$$

Then s^2_a may be called the *corrected mean square between columns*, and the numerator in equation (7.22D) is the *sum of squares between columns*. By means of equation (2B) the latter can be put into a form more convenient for computation, thus:

$$N_b \cdot {}^{N_a}S(\bar{y}_a - \bar{y})^2 = N_b \cdot \{{}^{N_a}S(\bar{y}^2_a) - N_a \cdot \bar{y}^2\}$$
$$= S_a - S_1, \text{ say,}$$

where

$$\left.\begin{aligned} S_a &= N_b \cdot {}^{N_a}S(\bar{y}^2_a) \\ &= {}^{N_a}S(\bar{y}_a[y_a]) \cdot \\ S_1 &= N \cdot \bar{y}^2 \\ &= [y] \cdot \bar{y} \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad (7.22E)$$

Thus to find S_a we take the total of each column, multiply it by the mean of

the same column, and add together all the products so formed. To find S_1 we multiply the grand total of the observations by the grand mean. Then

$$s^2{}_a = \frac{S_a - S_1}{N_a - 1} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.22F)$$

7.23 *The error component of the variance.* An estimate of the component σ_e^2 due to error can be made from any one column in the form :

$$\frac{{}^{N_b}S(y - \bar{y}_a)^2}{N_b - 1}$$

since the factor A contributes nothing to the variance within a column, being held constant throughout it ; the whole of the variation within a column is due to error factors. A more precise estimate can be got by averaging the above expression over all the N_a columns, thus :

$$s^2{}_e = \frac{{}^{N}S(y - \bar{y}_a)^2}{N_a(N_b - 1)} \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.23A)$$

The numerator in equation (7.23A) is called the *sum of squares within columns.* The number of degrees of freedom forming the denominator is seen to conform to the rule, § 2, since there are N observations y, and N_a different means $\bar{y}_a$ have been calculated from them for use in the equation, giving $(N - N_a)$ or $N_a(N_b - 1)$ degrees of freedom.

A formula better suited for computation is found thus, equation (2B) :

$$\begin{aligned} {}^{N}S(y - \bar{y})^2 &= {}^{N}S(y^2) - {}^{N_a}S([y_a] \cdot \bar{y}_a) \\ &= S_{ab} - S_a \end{aligned}$$

where

$$S_{ab} = {}^{N}S(y^2) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.23B)$$

for the purposes of §§ 7.2 and 7.3 only. (In § 7.4, S_{ab} has a different though analogous meaning). Hence

$$s^2{}_e = \frac{S_{ab} - S_a}{N_a(N_b - 1)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.23C)$$

7.24 *The analysis of variance.* When $s^2{}_a$ and $s^2{}_e$ have been computed, it is easy to deduce the value of $s^2{}_A$ and hence of s^2, equation (7.22C), but a different estimate s'^2 of σ^2 could be formed as follows :

$$s'^2 = \frac{{}^{N}S(y - \bar{y})^2}{N - 1} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.24A)$$

where the numerator ${}^{N}S(y - \bar{y})^2$ is called the *total sum of squares.* Being less precise than s^2, equation (7.22C), s'^2 is not used, but it gives rise to a relation known as the "analysis of variance." Thus the numerator in equation (7.24A) can be transformed as follows, from equation (2B) :

$$\left.\begin{aligned} {}^{N}S(y - \bar{y})^2 &= {}^{N}S(y^2) - \bar{y}[y] \\ &= S_{ab} - S_1. \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad (7.24B)$$

When this is compared with the numerators in equations (7.22F), (7.23C), we notice that

$$(S_{ab}-S_1)\equiv(S_{ab}-S_a)+(S_a-S_1) \quad . \quad . \quad . \quad . \quad (7.24C)$$

There is an analogous relation between the corresponding numbers of degrees of freedom which form the denominators in equations (7.24A), (7.23A) and (7.22F), namely (since $N=N_a.N_b$) :

$$(N_a.N_b-1)\equiv(N_a.N_b-N_a)+(N_a-1) \quad . \quad . \quad . \quad . \quad (7.24D)$$

Thus the total sum of squares has been divided into two component sums of squares in equation (7.24C), and the total degrees of freedom have been divided into two corresponding numbers of degrees of freedom in equation (7.24D). These two parallel analyses constitute an *analysis of variance*, although in fact it is the data for calculating variance that have been analysed, not the variance itself.

7.25 *Mnemonics for analyses of variance and covariance.* The terms of the following identity

$$ab-1\equiv(ab-a)+(a-1)$$

give the *suffixes for the symbols* S *and also for the corresponding symbols* N in equations (7.22D), (7.23C) and (7.24 A, C, D), if N_1 is a symbol for 1. Similar mnemonics are applicable to all analyses of variance and to the analogous symbols K used in the analysis of covariance, § 7.82 and Table 12. The correspondence between the suffixes affords a useful check and clue in such analyses, and possibly some inferences might be deduced from it.

7.26 *Test of significance.* The null hypothesis " that the factor A has no effect on the value of y " implies that σ^2_A is zero, and hence, equation (7.22AC), that s^2_a and s^2_e, equations (7.22D), (7.23A), are estimates of the same variance σ^2. The hypothesis can, therefore, be tested by writing, § 6.23 :

$$z=\tfrac{1}{2}\log_e(s_a^2/s^2_e)$$
$$n_1=(N_a-1)$$
$$n_2=N_a(N_b-1).$$

and if z is large enough to be significant, Figs. 69 (A, B), the factor A is declared to have a significant effect. The value of z can be read from the nomogram Fig. 68.

If the phases of A differ qualitatively (e.g. if each column corresponds to a given dose of a different drug) a t test may, provided that the z test has indicated significance, be applied as in § 6.22 to show whether there is a significant difference between the means $\bar{y}_a$ of any individual pair of columns. If, however, the pair under test has been picked out because it shows the largest of all the $\frac{1}{2}N_a(N_a-1)$ differences, a severer level of significance must be adopted for it since the chance that one or other of the differences shall exceed a specified value is greater than the chance that some one preselected difference shall do so. The test of significance in this case is a problem in the distribution of range ; see Kendall, vol. 1, sec. 9.21 ; table VI in Freeman's *Industrial Statistics* ; and Pearson's tables in *Biometrika*, **24**, 404, and **32**, 301.

7.27 *Regression.* If the effect of A is found to be significant, and if its levels **a** are measurable quantities, the magnitude of the response of y to a given change in A will be indicated, to a first approximation at all events, by a regression coefficient b_{ya} found thus, § 4.21 :

$$b_{ya}=\frac{{}^{N_a}S\{(\mathbf{a}-\bar{\mathbf{a}})(\bar{y}_a-\bar{y})\}}{{}^{N_a}S(\mathbf{a}-\bar{\mathbf{a}})^2}$$

If the levels of A are spaced at equal intervals $\delta\mathbf{a}$, then

$${}^{N_a}S(\mathbf{a}-\bar{\mathbf{a}})^2=\tfrac{1}{12}\,.\,(\delta\mathbf{a})^2\,.\,N_a(N^2{}_a-1)$$

so that

$$b_{ya}=\frac{12\,{}^{N_a}S\{(\mathbf{a}-\bar{\mathbf{a}})(\bar{y}_a-\bar{y})\}}{(\delta\mathbf{a})^2\,.\,N_a(N^2{}_a-1).}$$

The linearity of the regression can be tested as in § 6.25, and if necessary a transformation of coordinates can be effected, § 4.24, or a curvilinear regression can be formed, § 4.23.

Strictly such a regression applies only under the conditions obtaining in the experiment, and extrapolation to other conditions is justifiable only in so far as the former are representative of the latter.

7.3 Two balanced factors

In Table 5 let each row now correspond to a different phase or level **b** of a second factor B on which the magnitude of y depends (e.g. to the constitution of a different person B subjected to test). Then each phase of A will occur once in combination with each phase of B (e.g. each dosage of narcotic will be given once to each person on different occasions) so that correlation between A and B is eliminated. The factors A and B are then said to be *balanced.* Let $\bar{y}_b$ be the mean of any row.

Let $\sigma^2{}_e$ be the variance which y would have if both A and B were unvaried ; i.e. the variance due to error factors. Let $\sigma^2{}_A$ be the increment in the variance of y which would result if A received the values envisaged in Table 5 while B was held constant ; and let $\sigma^2{}_B$ have an analogous meaning. The effects measured by $\sigma^2{}_A$ and $\sigma^2{}_B$ are referred to as the *main effects* of A and B. It may happen, further, than when A and B are simultaneously varied through the phases envisaged in Table 5, the variance of y is still further increased by an amount $\sigma^2{}_{AB}$ as a result of *interaction* between A and B. (For example, a reaction time y of one person $\mathbf{b}_1$ may be more sensitive than that of another $\mathbf{b}_2$ to changes in dosage **a** of a narcotic.) Hence under the conditions supposed in Table 5 :

$$\sigma^2=\sigma^2{}_A+\sigma^2{}_B+\sigma^2{}_{ab} \quad . \ . \ . \ . \ . \ . \ . \quad (7.3A)$$

where

$$\sigma^2{}_{ab}=\sigma^2{}_e+\sigma^2{}_{AB} \quad . \ . \ . \ . \ . \ . \ . \quad (7.3B)$$

The interaction of A and B does not influence the deviation $(\bar{y}_a-\bar{y})$ of any column mean, because the whole of the effect of B has been averaged out of the latter, and similarly it does not affect the deviations $(\bar{y}_b-\bar{y})$ of any row mean ; but these deviations respectively go to make up the main effects of A and B. Hence if we subtract from any total deviate $(y-\bar{y})$ the deviates

for the means of the row and column in which it occurs, the residual deviate the effect of error and interaction alone. This residual deviate is

$$\{(y-\bar{y})-(\bar{y}_a-\bar{y})-(\bar{y}_b-\bar{y})\}, \text{ which}$$
$$=(y+\bar{y}-\bar{y}_a-\bar{y}_b)$$

and its variance is σ^2_{ab}.

In order to make an analysis of variance we write :

$$(y-\bar{y}) \equiv (\bar{y}_a-\bar{y})+(\bar{y}_b-\bar{y})+(y+\bar{y}-\bar{y}_a-\bar{y}_b) \quad . \quad . \quad . \quad (7.3C)$$

and square and sum both sides of the identity for the N observations y. Th result is : *

$$^{N}S(y-\bar{y})^2 \equiv N_b \,.\, ^{N_a}S(\bar{y}_a-\bar{y})^2+N_a \,.\, ^{N_b}S(\bar{y}_b-\bar{y})^2+{}^{N}S(y+\bar{y}-\bar{y}_a-\bar{y}_a)^2$$

which may be written :

$$(S_{ab}-S_1) \equiv (S_a-S_1)+(S_b-S_1)+(S_{ab}-S_a-S_b+S_1) \quad . \quad . \quad (7.3D)$$

where S_{ab}, S_a and S_1 have the same meanings as in § 7.2, and S_b is analogo to S_a, equations (7.22E).

For the main effects of A and B we can, as in equation (7.22F), write dow the estimates s^2_a, s^2_b, of the *distorted variances between columns and ro* respectively, thus :

$$\left.\begin{aligned} s^2_a &= \frac{S_a-S_1}{N_a-1} \\ s^2_b &= \frac{S_b-S_1}{N_b-1} \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.3$$

For the interaction term we have the sum of squares $(S_{ab}-S_a-S_b+S_1$ equation (7.3D), and it has $(N_a-1)(N_b-1)$ or $(N_aN_b-N_a-N_b+1)$ degrees freedom, as may be proved by the ordinary rule or by considering that wh the means of rows and columns are given, Table 5, any entries could be free

* *Proof.* For any one row there are N_a different quantities $(\bar{y}_a-\bar{y})^2$, and their sum $^{N_a}S(\bar{y}_a-\bar{y})^2$. These quantities and their sum are the same for each row, so that their tot sum is $N_b.^{N_a}S(\bar{y}_a-\bar{y})^2$. Column means similarly yield the term in $\bar{y}_b$.

We have also to prove that the cross products in the square of the right-hand si of equation (7.3C) sum up to zero. When the cross product $(\bar{y}_a-\bar{y})(\bar{y}_b-\bar{y})$ is summ in respect of the y's forming any one column, $\bar{y}_b$ varies from row to row, while $\bar{y}_a$ is t same throughout the column. Hence

$$^{N_b}S(\bar{y}_a-\bar{y})(\bar{y}_b-\bar{y}) = (\bar{y}_a-\bar{y}) \,.\, ^{N_b}S(\bar{y}_b-\bar{y}) = 0$$

since $\bar{y}$ is the mean of the row means $\bar{y}_b$. Again

$$(y-\bar{y}_a-\bar{y}_b+\bar{y}) \equiv (y-\bar{y})-(\bar{y}_a-\bar{y})-(\bar{y}_b-\bar{y}).$$

Consider the cross product of $(\bar{y}_a-\bar{y})$ with each of the three brackets on the right, a sum throughout any one column. Then for the first bracket

$$^{N_b}S(\bar{y}_a-\bar{y})(y-\bar{y}) = N_b(\bar{y}_a-\bar{y})^2$$

which cancels with the sum of cross products for the second bracket. The cross produ for the last bracket has already been shown to sum up to zero.

Similarly the cross product of $(\bar{y}_b-\bar{y})$ with each of the three brackets sums up to ze for each row.

made in (N_a-1) columns and (N_b-1) rows without producing inconsistency, provided that suitable entries were made in the outstanding column and row. Hence for an estimate of the interaction-and-error variance σ^2_{ab} we have :

$$s^2_{ab}=\frac{S_{ab}-S_a-S_b+S_1}{N_a.N_b-N_a-N_b+1} \qquad . \ . \ . \ . \ . \quad (7.3F)$$

It is impossible with a single set of observations corresponding to all the combinations of levels to separate s^2_{AB} from s^2_e ; they are confounded in the term s^2_{ab}. They could, however, be separated by replicating the experiment and treating each replication or set of observations as corresponding to one phase of a third factor C, as in § 7.4.

For the analysis of variance the mnemonic giving the suffixes of S and N is :

$$ab-1\equiv(a-1)+(b-1)+(ab-a-b+1).$$

Thus for the sums of squares :

$$S_{ab}-S_1\equiv(S_a-S_1)+(S_b-S_1)+(S_{ab}-S_a-S_b+S_1)$$

and for the degrees of freedom :

$$N_a.N_b-1\equiv(N_a-1)+(N_b-1)+(N_a.N_b-N_a-N_b+1)$$
$$N=N_a.N_b.$$

If the null hypothesis " that the effect of A is negligible " is true, then σ^2_A will be negligible and the interaction σ^2_{AB} also will be negligible (though in the rare case of catalysis the main effect alone of A might be negligible). Then s^2_a and s^2_{ab} will both be estimates of the same variance, namely that, σ^2_e, which is attributable to the residual error, so that for a test of significance, equations (7.3E, 7.3F) :

$$z=\tfrac{1}{2}\log_e(s^2_a/s^2_{ab})$$
$$n_1=(N_a-1)$$
$$n_2=(N_a-1)(N_b-1)$$

and an analogous test is applicable to the significance of the effects of B.

4 Three or more balanced factors with complete set of factorial combinations

Let a rectangular two-factor experiment, § 7.3 and Table 5, be repeated N_c times, and in each repetition let a different phase or level **c** of some third factor be conjoined with each pair of phases **a** and **b** of A and B. (For example, the levels of C may be degrees of fatigue caused by different measured amounts of exertion, while A still measures a dose of narcotic, the phases of B are different persons under test, and y measures a specified reaction time.) Then the total number of observations

$$N=N_a.N_b.N_c$$

and Table 5 becomes a plan view of a three-dimensional table in which a pile of N_c cubic cells stands on every square y_{ab}, each cell in one pile being labelled

with a different phase or level **c** of C but with the same pair **a**, **b** of phases o levels of A and B. Let the sum of the N_c observations y_{ab} in one such pil be denoted by $[y_{ab}]$ and their mean $[y_{ab}]/N_c$ by $\bar{y}_{ab}$. There will be $N_a.N_b$ suc sums and means, and the same number of products $\bar{y}_{ab} . [y_{ab}]$. Let thes $N_a.N_b$ products be summed, let their sum be denoted by S_{ab}, and let S_{bc}, S_{ca} have analogous meanings.

As before let $[y_a]$ denote the sum of all the observations y or y_a that corre spond to one of the levels **a** of A. There are now $N_b.N_c$ observations at tha level, so that the mean $\bar{y}_a$ for the level will be $[y_a]/N_b.N_c$. The product o sum and mean for the same level is $[y_a] . \bar{y}_a$ and there are N_a such product Let the sum of the N_a products be denoted by S_a, and let S_b, S_c have analogou meanings.

Let $^{N}S(y^2)$ be denoted by S_{abc}. It will readily be seen that if there wer four balanced factors A, B, C, D, then the appropriate symbol for $S(y^2)$ woul be S_{abcd}. The sum $S(y^2)$ is always denoted by the S with suffix of highest orde because there is only one observation in each of the finest sub-classes or sing cells into which the data are cross-classified.

The analysis of variance is set out in Table 6, and it will be seen that th sums of squares and degrees of freedom add up to the totals in the usual wa that the suffixes of N correspond to those of S, and that there are now thre different interaction sums of the first order. The quantity :

$$(S_{abc}-S_{ab}-S_{bc}-S_{ca}+S_a+S_b+S_c-S_1)$$

is called the *interaction sum of squares of the second order*. Its corrected mea square, which will be denoted by s^2_{abc}, may be used as an error term for testin such null hypotheses as " that the main effect of A is negligible " or " that th interaction of A and B is negligible," since the mean square due to error confounded in it with the mean square due to the second-order interactio which is usually small. Thus

$$s^2_{abc}=\frac{S_{abc}-S_{ab}-S_{bc}-S_{ca}+S_a+S_b+S_c-S_1}{(N_a-1)(N_b-1)(N_c-1)}$$

$$s^2_a=\frac{S_a-S_1}{N_a-1}$$

$$s^2_{ab}=\frac{S_{ab}-S_a-S_b+S_1}{(N_a-1)(N_b-1)}$$

For testing the significance of the main effect of A,

$$z=\tfrac{1}{2}\log_e(s^2_a/s^2_{abc})$$
$$n_1=N_a-1$$
$$n_2=(N_a-1)(N_b-1)(N_c-1)$$

For testing the significance of the interaction AB :

$$z=\tfrac{1}{2}\log_e(s^2_a/s^2_{abc})$$
$$n_1=(N_a-1)(N_b-1)$$
$$n_2=(N_a-1)(N_b-1)(N_c-1).$$

TABLE 6. ANALYSIS OF VARIANCE FOR A COMPLETE REPLICATION COMPRISING N OBSERVATIONS y INFLUENCED BY THREE BALANCED FACTORS A, B, C HAVING N_a, N_b, N_c LEVELS OR PHASES RESPECTIVELY, § 7.4

$$N = N_a.N_b.N_c \qquad S_1 = [y].\bar{y} = N.\bar{y}^2 \qquad S_{abc} = [y^2]$$

$$S_a = {}^{N_a}S([y_a].\bar{y}_a) \qquad S_{ab} = {}^{N_a.N_b}S([y_{ab}].\bar{y}_{ab})$$

Nature of variation	Sum of squares: Basic formula	Sum of squares: Formula for computation	Mnemonic for signs and suffixes of S and N	Degrees of freedom	Corrected mean square
Main effects:					
A	$N_b.N_c\,.\,{}^{N_a}S(\bar{y}_a-\bar{y})^2$	S_a-S_1	$a-1$	N_a-1	s^2a
B	$N_c.N_a\,.\,{}^{N_b}S(\bar{y}_b-\bar{y})^2$	S_b-S_1	$b-1$	N_b-1	s^2b
C	$N_a.N_b\,.\,{}^{N_c}S(\bar{y}_c-\bar{y})^2$	S_c-S_1	$c-1$	N_c-1	s^2c
First-order interactions:					
AB	$N_c.{}^{N_a.N_b}S(\bar{y}_{ab}-\bar{y}_a-\bar{y}_b+\bar{y})^2$	$S_{ab}-S_a-S_b+S_1$	$ab-a-b+1=(a-1)(b-1)$	$(N_a-1)(N_b-1)$	s^2ab
BC	$N_a.{}^{N_b.N_c}S(\bar{y}_{bc}-\bar{y}_b-\bar{y}_c+\bar{y})^2$	$S_{bc}-S_b-S_c+S_1$	$c-b-c+1=(b-1)(c-1)$	$(N_b-1)(N_c-1)$	s^2bc
CA	$N_b.{}^{N_c.N_a}S(\bar{y}_{ca}-\bar{y}_c-\bar{y}_a+\bar{y})^2$	$S_{ca}-S_c-S_a+S_1$	$ca-c-a+1=(c-1)(a-1)$	$(N_c-1)(N_a-1)$	s^2ca
Second-order interaction:					
ABC	${}^{N}S(y-\bar{y}_{ab}-\bar{y}_{bc}-\bar{y}_{ca}+\bar{y}_a+\bar{y}_b+\bar{y}_c-\bar{y})^2$	$S_{abc}-S_{ab}-S_{bc}-S_{ca}+S_a+S_b+S_c-S_1$	$abc-ab-bc-ca+a+b+c-1=(a-1)(b-1)(c-1)$	$(N_a-1)(N_b-1)(N_c-1)$	s^2abc
Total . .	${}^{N}S(y-\bar{y})^2$	$S_{abc}-S_1$	$abc-1$	$N-1$, i.e. $N_a.N_b.N_c-1$	—

If the first-order interactions are found to be insignificant, a more sensitiv test for the main effect of A can be obtained by lumping together all fou interaction terms and using the resulting corrected mean square s^2_i instea of s^2_{abc} so as to have more degrees of freedom, thus :

$$s^2_i = \frac{S_{abc} - S_a - S_b - S_c + 2S_1}{N_a.N_b.N_c - N_a - N_b - N_c + 2}$$

$$z = \tfrac{1}{2} \log_e (s^2_a / s^2_i)$$

$$n_1 = N_a - 1$$

$$n_2 = N - N_a - N_b - N_c + 2.$$

If there were four or more balanced factors A, B, C, D . . . the formul in Table 6 could be extended in an obvious manner. However, if each facto has more than two levels the number of observations quickly becomes ver great, and in practice it is necessary to use a contracted design, § 7.6, especial in experiments which impose any degree of hardship on the animals employed

7.5 Analysis by orthogonal functions

In equations like equation (7.24B) the numbers of squares included in th sums of squares are not the same as the numbers of degrees of freedom. In a alternative mode of analysis, however, the total sum is partitioned into (N− squares $(Z/h)^2$, each of which has 1 degree of freedom. Thus :

$$^{N}S(y-\bar{y})^2 = \frac{Z_1^2}{h_1^2} + \frac{Z_2^2}{h_2^2} + \frac{Z_3^2}{h_3^2} + \ldots + \frac{Z^2_{N-1}}{h^2_{N-1}} \quad . \quad . \quad . \quad (7.5A)$$

Each function Z is a linear function of the N observations y, which are he numbered from 1 to N, so that if the quantities l are a constants :

$$\left.\begin{aligned} Z_1 &= l_{11}y_1 + l_{12}y_2 + l_{13}y_3 \ldots l_{1N}y_N \\ Z_2 &= l_{21}y_1 + l_{22}y_2 + l_{23}y_3 \ldots l_{2N}y_N \\ &\cdots\cdots\cdots\cdots \\ Z_k &= l_{k1}y_1 + l_{k2}y_2 + l_{k3}y_3 \ldots l_{kN}y_N \\ &\cdots\cdots\cdots\cdots \\ Z_{N-1} &= l_{N-1,1}y_1 + l_{N-1,2}y_2 + l_{N-1,3}y_3 \ldots l_{N-1,N}y_N \end{aligned}\right\} \quad . \quad . \quad (7.5B)$$

In order that equations (7.5A) and (7.5B) may be satisfiable the following co ditions must hold, where k and m stand for any whole numbers up to (N−1)

For each function Z_k

$$l_{k1} + l_{k2} + l_{k3} \ldots + l_{kN} = 0 \quad . \quad . \quad . \quad . \quad . \quad (7.5C)$$

$$h^2_k = l^2_{k1} + l^2_{k2} + l^2_{k3} \ldots l^2_{kN} \quad . \quad . \quad . \quad . \quad (7.5D)$$

For each pair of functions Z_k, Z_m

$$l_{k1} \cdot l_{m1} + l_{k2} \cdot l_{m2} + l_{k3} \cdot l_{m3} \ldots + l_{kN} \cdot l_{mN} = 0 \quad . \quad . \quad (7.5E)$$

Various sets of functions Z could be chosen, but in the preferred forms th constants have simple values such as 0, ±1 or ±2. Such functions can b generated as follows in the case in which each of three factors A, B and C ha

two levels, such as presence and absence, and one observation is taken at each combination of levels. Let a stand for the observation y obtained when A alone is present, ab for that when A and B are present without C, and so on, while the numeral 1 stands for the observation obtained in the absence of all three factors, i.e. a control observation. (The numeral 1 is here a label for the observation, not a measure of it.) The symbols a, ab, abc . . . are used, with harmless ambiguity, sometimes for the observations and sometimes for the corresponding *treatments*, or combinations of factor-levels, that respectively give rise to them. The complete set of eight observations is denoted in the expansion of $[y]$ where :

$$[y]=(a+1)(b+1)(c+1)$$
$$=abc+ab+bc+ca+a+b+c+1 \quad . \quad . \quad . \quad . \quad (7.5\text{F}).$$

There are four observations at each of the two levels of A, which levels may be called **a** and α respectively. Let $[y_a]$ and $[y_\alpha]$ denote the sums of these two sets of observations and let a symbol (A), called a *comparison* or *contrast* and serving in some sort as a measure of the main effect of A, be defined thus :

$$(\text{A})=[y_a]-[y_\alpha]$$
$$=(abc+ab+ca+a)-(bc-b+c+1) \quad . \quad . \quad . \quad . \quad (7.5\text{G})$$

which is an expansion of the symbolical mnemonic :

$$(a-1)(b+1)(c+1).$$

Similarly for interactions of the first and second orders :

$$(\text{AB})=(a-1)(b-1)(c+1)$$
$$=(abc+ab+c+1)-(a+b+bc+ca) \quad . \quad . \quad . \quad . \quad (7.5\text{H})$$
$$(\text{ABC})=(a-1)(b-1)(c-1)$$
$$=(abc+a+b+c)-(ab+bc+ca+1) \quad . \quad . \quad . \quad . \quad (7.5\text{I}).$$

The other four comparisons have analogous forms.

The seven comparisons (ABC), (AB), (BC), (CA), (A), (B), (C), are a set of orthogonal functions Z, equation (7.5A), and in each case $h^2=8$ by equation (7.5D) ; for instance if a is y_1 and (A) is Z_1, l_{11} is $+1$ in equation (7.5B) ; each is $+1$ or -1 and there are eight of them for each Z.

The observations a, b, c may be the yields of three plots of land treated respectively with three fertilizers, such as lime, potash and nitrate, while the remaining five observations are the yields of a control plot and of four plots treated with different combinations of these fertilizers. The experiment may be replicated by applying the same set of treatments to eight plots arranged in random order in a second block of land, and so on for three or more blocks. The error component of the variance arises from unavoidable differences in fertility, etc. between the plots within a block. Greater and systematic differences, however, are to be expected between one block and another, and the effects of these are eliminated from the error by treating each block as a different phase of a new factor R and including this factor in the analysis of variance. Besides main effects of

blocks there may be interactions of blocks with treatments ; that is, some blocks may be more favourable to the effects of one treatment than of others.

In a feeding experiment, the treatments might comprise the addition of three different vitamins to the diet, in all possible combinations ; instead of the plots there would be experimental animals or persons, and a block would comprise observations on a set of approximately homogeneous animals or persons such as animals of the same litter or persons similar in age and sex.

Let the experiment be replicated N_r times so that there are N_r blocks R each corresponding to a complete set of eight factorial combinations or treatments and so containing eight observations ; and let *a* now stand for the sum of the N_r observations under treatment *a*, while *ab*, *abc*, etc., have analogous meanings for treatments *ab*, *abc*, etc. (Thus the previous meanings still hold when $N_r=1$.) Then the comparisons (A), (AB), etc., as defined by equations (7.6 G, H, I) are still orthogonal functions Z with one degree of freedom but each of them now contains $8N_r$ terms and for each, therefore, equation (7.5D), since every *l* is ± 1 :

$$h^2=8N_r.$$

Moreover, since there are now $8N_r$ observations, the total number of degrees of freedom is $(8N_r-1)$ instead of 7, and the balance is made up by $(8N_r-8)$ degrees of freedom for the effects of blocks, *each block being treated as if it corresponded to a different phase of some new factor R.* Then from equation (7.5A) :

$$^{N}S(y-\bar{y})^2=\frac{(ABC)^2}{8N_r}+\frac{(AB)^2}{8N_r}+\frac{(BC)^2}{8N_r}+\frac{(CA)^2}{8N_r}+\frac{(A)^2}{8N_r}+\frac{(B)^2}{8N_r}+\frac{(C)^2}{8N_r}+S_R\ . \quad (7.5J)$$

where S_R is the sum of squares, with $(8N_r-8)$ degrees of freedom, for the main effects of the replications or blocks and for their interactions with treatments and disappears when $N_r=1$.

For a test of significance, the sum of squares for the main effect A comprises a single square, $(A)^2/8N_r$ with one degree of freedom. This follows from equations (7.5A, C, D, E) or from equation (7.5G) coupled with an extension of Table 6 to four factors. Thus, Table 6 :

$$N_a=N_b=N_c=2\ ;$$

$$N=N_a.N_b.N_c.N_r=8N_r;$$

$$S_a-S_1=\left(\frac{[y_a]^2}{4N_r}+\frac{[y_\alpha]^2}{4N_r}\right)-\left(\frac{[y_a+y_\alpha]^2}{8N_r}\right)$$

since $\bar{y}_a=[y_a]/4N_r$, $\bar{y}_\alpha=[y_\alpha]/4N_r$, and $\bar{y}=[y_a+y_\alpha]/8N_r$

Hence $S_a-S_1=([y_a]-[y_\alpha])^2/8N_r$

$=(A)^2/8N_r$

Since there is only one degree of freedom the same expression is a corrected mean square ; thus, equation (7.3E) :

$$s^2_a=(A)^2/8N_r$$

and similarly the corrected mean squares for the interactions are computed on the following models :

$$s^2_{ab}=(AB)^2/8N_r\,;\quad s^2_{abc}=(ABC)^2/8N_r.$$

The sum of squares S_R shown in equation (7.5J) as arising from the assortments or blocks has $(8N_r-8)$ degrees of freedom, for 1 out of the total of $(8N_r-1)$ goes to each of the seven treatments. Further, (N_r-1) degrees must be allocated to the main effect of differences between the N_r blocks, so that $7(N_r-1)$ are left for the interactions between blocks and treatments, which constitute the residual error. This number is clearly obtained thus :

$$\begin{aligned}7(N_r-1)&=(N_a.N_b.N_c-1)(N_r-1)\\&=N_a.N_b.N_c.N_r-N_a.N_b.N_c-N_r+1.\end{aligned}$$

To find the sum of squares for this residual error the same suffixes must be assigned to the symbols S as to the symbols N. Let the corresponding estimate of variance be denoted by s^2_{ri}. Then

$$s^2_{ri}=\frac{S_{abcr}-S_{abc}-S_r+S_1}{7(N_r-1)}$$

where $S_{abcr}=[y^2]$, the sum of the squares of all the observations ;

$S_{abc}=N_r\,.\,{}^8S(\bar{y}^2_{abc})$;

$S_r=8\,.\,{}^{N_r}S(\bar{y}^2_r)$;

$S_1=8N_r\,.\,\bar{y}^2$, viz. $8N_r$ times the square of the grand mean.

$\bar{y}_{abc}$ denotes the mean of the N_r observations which correspond to any one of the eight treatments. These eight means are squared, the squares are summed, and the sum is multiplied by N_r, the number of blocks, to yield S_{abc}. Similarly $\bar{y}_r$ is the mean of the eight observations in any block, and S_r is eight times the sum of the squares of these N_r means. For tests of significance :

$$t_{[7N_r-7]}=\frac{s_a}{s_{ri}},\text{ or }\frac{s_{ab}}{s_{ri}},\text{ or }\frac{s_{abc}}{s_{ri}},\text{ or the like.}$$

The foregoing discussion, which is given in order to clarify the theory, implies the use of complete sets of factorial combinations with large numbers of observations. In practice, however, the number of observations will be reduced by contraction, § 7.6, with confounding, § 7.7.

7.6 Contracted designs for three or more balanced factors

A set of observations corresponding to all combinations of all the levels of the balanced factors would, if there were five factors having five levels each, comprise 3,125 observations, but the main effects which it indicates can be ascertained, with a smaller but possibly adequate degree of precision, from a single block of 25 observations if the block is planned as a balanced factorial square.

If, with three controlled factors A, B and C,

$$N_a=N_b=N_c=\sqrt{N}$$

then with only N observations each level of each factor can be combined once

with each level of each other factor, though not with every combination of those levels ; i.e. the design can be fully balanced in respect of main effects, but must be randomized in respect of interactions. Such an arrangement is a *Latin square*, and an example for which N=9 is given in Table 7. A complete set of all possible combinations of levels would consist of $\sqrt{N}$ different Latin squares or blocks so designed that each could be derived as in Table 8 from any other, by cyclic interchange of the levels of one factor such as C. Different sets of factorial combinations, each comprising $\sqrt{N}$ squares cyclically related in respect of C, can be formed by interchange of levels of A and of B. In practice, however, one or two blocks designed as Latin squares may give adequate precision, a complete set of combinations involving a wasteful use of animals, § 9.3.

TABLE 7 (A). THE SAME LATIN SQUARE TABULATED IN THREE DIFFERENT WAYS

Nine different combinations are formed by taking the letter in each square and combining it with the two letters level with it in the margins.

	a_1	a_2	a_3
b_1	c_1	c_2	c_3
b_2	c_2	c_3	c_1
b_3	c_3	c_1	c_2

	c_1	c_2	c_3
a_1	b_1	b_2	b_3
a_2	b_3	b_1	b_2
a_3	b_2	b_3	b_1

	b_1	b_2	b_3
c_1	a_1	a_3	a_2
c_2	a_2	a_1	a_3
c_3	a_3	a_2	a_1

TABLE 7 (B). TWO DIFFERENT LATIN SQUARES DERIVED FROM TABLE 7 (A) BY CYCLIC INTERCHANGE OF LEVELS OF C

	a_1	a_2	a_3
b_1	c_2	c_3	c_1
b_2	c_3	c_1	c_2
b_3	c_1	c_2	c_3

	a_1	a_2	a_3
b_1	c_3	c_1	c_2
b_2	c_1	c_2	c_3
b_3	c_2	c_3	c_1

TABLE 8. A GRÆCO-LATIN SQUARE FOR FOUR FACTORS A, B, C, D AT 3 LEVELS EACH

	a_1	a_2	a_3
b_1	c_1 d_3	c_2 d_2	c_3 d_1
b_2	c_2 d_1	c_3 d_3	c_1 d_2
b_3	c_3 d_2	c_1 d_1	c_2 d_3

A fourth factor can have each of its phases conjoined once with each of the phases of A, of B and of C in the same block, forming a *Græco-Latin square* (Table 8), and similarly various numbers of factors up to $(\sqrt{N}-1)$ can be arranged factorially in a single balanced block, provided that N has certain values. A number of factorial-square schematics are given in Fisher and Yates's Tables XV and XVI.

If N_b and N_c are equal to one another but unequal to N_a, then each phase of B and of C can be combined with each phase of A, but a selection has to be made out of the possible combinations of B with C. This is done by lot : for instance all the levels of C could be entered in each row of Table 5 in random order. Such an arrangement is a *randomized block*, in which A is balanced with B and with C, and B is randomized with C but not balanced with it. Fisher has shown that the substitution of any systematic arrangement for randomization of unbalanced factors is likely to falsify the estimate of error.

The analysis of a single Latin square with three factors at three levels each is given in Table 9. Interactions are confounded with error unless the experiment is replicated. The increase in precision and sensitivity which is gained by removing the effects of regulated factors from among those of the error factors will be readily appreciated. Significance is tested by using the residual mean square s^2_r as a measure of error, thus :

$$z=\tfrac{1}{2}\log_e(s^2_a/s^2_r)$$
$$n_1=\sqrt{N}-1=2$$
$$n_2=(N-1)-3(\sqrt{N}-1)=2.$$

For larger values of N, the numeral 2 must be replaced throughout Table 9 by $(\sqrt{N}-1)$, and additional symbols such as S_d, S_e . . . and N_d, N_e . . . must be included.

TABLE 9. ANALYSIS OF VARIANCE FOR A LATIN SQUARE WITH THREE FACTORS A, B, C AT THREE LEVELS EACH, §§ 7.4, 7.6

$S_a=\sqrt{N}S([y_a]\cdot\bar{y}_a)$ $\quad$ $S_1=[y]\cdot\bar{y}$

$N_a=N_b=N_c=\sqrt{N}=3$ $\quad$ $S_N=S_{ab}=S_{bc}=S_{ca}=S(y^2)$

Factor	Sum of squares	Degrees of freedom	Corrected mean square
A . . .	S_a-S_1	$N_a-1=2$	$s^2_a=\dfrac{S_a-1}{N_a-1}$
B . . .	S_b-S_1	$N_b-1=2$	$s^2_b=\dfrac{S_b-1}{N_b-1}$
C . . .	S_c-S_1	$N_c-1=2$	$s^2_c=\dfrac{S_c-1}{N_c-1}$
Residual error.	$S_N-S_a-S_b-S_c+2S_1$	$N-N_a-N_b-N_c+2=2$	$s^2_r=\dfrac{S_N-S_a-S_b-S_c+2S_1}{N-N_a-N_b-N_c+2}$
Total . .	S_N-S_1	$N-1=8$	—

7.7 Confounding

The number of observations required for a given degree of precision can be reduced by confounding. If two disturbing factors B and D are not of intrinsic interest they can be *confounded* by always pairing together the same levels of B and D ; this is the antithesis of randomization. Then in the analysis of variance the effects of B and D can together be eliminated from those of error, but cannot be distinguished from one another. (For example, if each person **b**, § 7.4, is kept on a different diet **d**, then the effects of personal constitution B are confounded with those of diet D.)

If the eight observations in equation (7.5I) are divided into two sub-blocks comprising respectively the terms with positive and with negative signs, and if each sub-block is replicated N_r times, then the comparison (ABC) is confounded with comparisons between the two sets of sub-blocks. This device is advantageous when the experimental conditions can be kept uniform throughout blocks of four but not of eight observations, provided that the effect (ABC) is not of intrinsic interest ; for some fluctuations (main effects between sub-blocks) which would otherwise go to swell the residual error (interactions of blocks with treatments) are now extracted in the additional N_r comparisons between sub-blocks, Table 10.

TABLE 10. CONFOUNDING IN N_r REPLICATIONS WITH

Treatments confounded with blocks→	None	
(No. of blocks) × (No. of treatments per block)→	$N_r \times 8$	
Allocation of treatments to blocks→	(*abc*, *ab*, *bc*, *ca*, *a*, *b*, *c*, 1)	
Comparisons ↓	Sums of squares	Degrees of freedom
(A)	$(A)^2/8N_r$	1
(B)	$(B)^2/8N_r$	1
(C)	$(C)^2/8N_r$	1
(AB)	$(AB)^2/8N_r$	1
(BC)	$(BC)^2/8N_r$	1
(CA)	$(CA)^2/8N_r$	1
(ABC)	$(ABC)^2/8N_r$	1
Between blocks or sub-blocks	$^{N_r}S([y_r]\bar{y}_r]-[y]\,.\,\bar{y}$	N_r-1
Residual error	Residue	$7(N_r-1)$
Total	$[y^2]-[y]\,.\,\bar{y}$	$8N_r-1$

Alternatively let there be four kinds of sub-blocks with two treatments in each, arranged as follows and lettered F, G, H, J :

F, (*abc* and *a*) ; G, (*ab* and *ca*) ;
H, (*b* and *c*) ; J, (*bc* and 1).

Then

$$\begin{aligned}(A) &= (abc+a+ab+ca)-(bc+b+c+1)\\ &= (F+G)-(J+H)\\ (BC) &= (abc+bc+a+1)-(b+c+ab+ca)\\ &= (F+J)-(G+H)\\ (ABC) &= (abc+a+b+c)-(ab+bc+ca+1)\\ &= (F+H)-(G+J)\end{aligned}$$

Thus the comparisons (A), (BC) and (ABC) are all confounded with comparisons between different kinds of sub-blocks.

When several comparisons between treatments are confounded with comparisons between blocks in this way, the symbol for each of the treatment comparisons which are confounded, such as (A), (BC) and (ABC), will be found to have an even number of letters in common with those treatment symbols, in this case *bc*, which cohabit a sub-block with the control symbol 1, zero

THREE FACTORS A, B, C, EACH AT TWO LEVELS

ABC		ABC, BC and A	
$2N_r \times 4$		$4N_r \times 2$	
(*abc*, *a*, *b*, *c*) ; (*ab*, *bc*, *ca*, 1)		(*abc*, *a*) ; (*ab*, *ca*) ; (*b*, *c*) ; (*bc*, 1)	
Sums of squares	Degrees of freedom	Sums of squares	Degrees of freedom
$(A)^2/8N_r$	1	—	—
$(B)^2/8N_r$	1	$(B)^2/8N_r$	1
$(C)^2/8N_r$	1	$(C)^2/8N_r$	1
$(AB)^2/8N_r$	1	$(AB)^2/8N_r$	1
$(BC)^2/8N_r$	1	—	—
$(CA)^2/8N_r$	1	$(CA)^2/8N_r$	1
—	—	—	—
$^{2N_r}S([y_{r/2}] \cdot \bar{y}_{r/2}) - [y] \cdot \bar{y}$	$2N_r - 1$	$^{4N_r}S([y_{r/4}] \cdot \bar{y}_{r/4}) - [y] \cdot \bar{y}$	$4N_r - 1$
Residue	$6(N_r - 1)$	Residue	$4(N_r - 1)$
$[y^2] - [y] \cdot \bar{y}$	$8N_r - 1$	$[y^2] - [y] \cdot \bar{y}$	$8N_r - 1$

counting as an even number. By this rule the confounded comparisons can be ascertained when there are more than three factors.

With factors at more than two levels the interactions can be partially confounded with error by forming the blocks as Latin squares cyclically related with respect to one of the factors.

7.8 Analyses involving covariance

7.81 *Taking out a linear regression.* If a linear regression of y on x is formed as in § 4.21 and if b is written for b_{yx}, then

$$Y-\bar{y}=b(x-\bar{x}) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.81A)$$

Now instead of reckoning deviations from the mean $\bar{y}$ we may reckon them from the regression line, so that a deviation is now $(y-Y)$ instead of $(y-\bar{y})$.

But $$y-\bar{y}=(y-Y)+(Y-\bar{y})$$

and when this equation is squared and summed the cross products sum up to zero *.

Thus $$^{N}S(y-\bar{y})^2={}^{N}S(y-Y)^2+{}^{N}S(Y-\bar{y})^2 \quad . \quad . \quad . \quad . \quad . \quad (7.81B)$$

But $^{N}S(Y-\bar{y})^2=b^2 \,.\, {}^{N}S(x-\bar{x})^2$, equation (7.81A)

$=r^2 \,.\, {}^{N}S(y-\bar{y})^2$, § 4.31, where r is the interclass correlation coefficient.

Hence $$^{N}S(y-Y)^2=(1-r^2).{}^{N}S(y-\bar{y})^2.$$

Therefore $$\frac{^{N}S(Y-\bar{y})^2}{^{N}S(y-Y)^2}=\frac{r^2}{1-r^2}$$

The numerator represents the effect of regression and is zero, except for random error, if the value of y is unresponsive to changes in x; and it has one degree of freedom, since a straight line through the point $(\bar{x}, \bar{y})$ can be made to pass through any one other point. The denominator has $(N-2)$ degrees of freedom (viz. N less 1 for $\bar{y}$ and 1 for b) and measures the error of y purified from the effect of regression. Hence if Student's t, § 3.33, is formed thus

$$t_{[N-2]}=\sqrt{\frac{(N-2)r^2}{1-r^2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.81C)$$

a large value of t indicates that the apparent linear regression and correlation are significant and not attributable to random errors.

This theorem can be used to subdivide equation (7.24A) in the manner shown in Table 11, and provides a test for the linearity of a regression, § 6.25. A similar subdivision can be made of corresponding terms in such analyses as that given in Table 6.

7.82 *The analysis of covariance.* Let a second dependent variate x in a complete set of factorial combinations when there are three balanced factors, § 7.4, be conjoined with y; then a value of y will be entered along with a value

* *Proof.*

$$(y-Y)(Y-\bar{y})=\{(y-\bar{y})-b(x-\bar{x})\} \,.\, b(x-\bar{x})$$
$$\therefore {}^{N}S(y-Y)(Y-\bar{y})=b\{{}^{N}S(y-\bar{y})(x-\bar{x})-b \,.\, {}^{N}S(x-\bar{x})^2\}$$
$$=0 \text{ by equation (4.21B).}$$

of x in each cell of Table 5. (For example, x might be the score in an intelligence test while y is a reaction time as in § 7.4.)

The sum of products

$$^{N}S(y-\bar{y})(x-\bar{x}) = {}^{N}S(xy) - N\bar{x}\bar{y}.$$

Write

$$N\bar{x}\bar{y} = \bar{x} \,.\, [y] = [x] \,.\, \bar{y}$$
$$= K_1, \text{ say, by analogy with equation (7.22E).}$$

Let $[x_a]$ be the sum of the $N_b.N_c$ different observations x_a which correspond to a level **a** of a factor A, and let $\bar{x}_a$ be their mean. Then, with analogous expressions for y,

$$N_b.N_c.\bar{x}_a\bar{y}_a = [x_a].\bar{y}_a = \bar{x}_a.[y_a].$$

Write

$$K_a = {}^{N_a}S(\bar{x}_a.[y_a])$$

so that K_a is the sum of the N_a products that can be formed by taking a different level or phase **a** for each product. Let K_b, K_c, have analogous meanings.

TABLE 11. ANALYSIS OF VARIANCE WITH A REGRESSION OF y ON **a** TAKEN OUT, § 7.81, FOR N PAIRS OF OBSERVATIONS ARRANGED IN N_a ARRAYS.

The mean of any array is denoted by $\bar{y}_a$ and the number of observations in it by N_b. Hence

$$N = {}^{N_a}S(N_b),$$
$$= N_a.N_b \text{ if } N_b \text{ is constant.}$$

Variation		Sum of squares		Degrees of freedom	
Within arrays→		$^{N}S(y-\bar{y}_a)^2$		$N-N_a$	
Between arrays	Deviations from regression →	$^{N_a}S\{N_b(\bar{y}_a-\bar{y})^2\}$	$^{N_a}S\{N_b(\bar{y}_a-Y)^2\}$	N_a-1	N_a-2 for linear regression
	Regression→		$^{N_a}S\{N_b(Y-\bar{y})^2\}$		1 for linear regression
Total		$^{N}S(y-\bar{y})^2$		$N-1$	

Let $[x_{ab}]$ and $[y_{ab}]$ be the respective totals of the N_c different observations x_{ab}, y_{ab} which belong to one combination of levels **a**, **b** of A and B, and let $\bar{x}_{ab}$, $\bar{y}_{ab}$ be their means. Then

$$N_c \,.\, \bar{x}_{ab} \,.\, \bar{y}_{ab} = \bar{x}_{ab} \,.\, [y_{ab}] = [x_{ab}] \,.\, \bar{y}_{ab}.$$

Write

$$K_{ab} = {}^{N_a \,.\, N_b}S\{[x_{ab}] \,.\, \bar{y}_{ab}\}$$

so that K_{ab} is the sum of the $N_a.N_b$ products that can be formed by choosing different combinations of the levels **a** and **b**. Let K_{bc}, K_{ca} have analogous meanings. For the sum of highest order it is clear by analogy that, when there are three factors :

$$K_{abc} = {}^{N}S(xy).$$

Write

$$(x-\bar{x}) \equiv (\bar{x}_a-\bar{x}) + (\bar{x}_b-\bar{x}) + (\bar{x}_c-\bar{x}) + R_x$$
$$(y-\bar{y}) \equiv (\bar{y}_a-\bar{y}) + (\bar{y}_b-\bar{y}) + (\bar{y}_c-\bar{y}) + R_y$$

TABLE 12. ANALYSIS OF COVARIANCE FOR TWO DEPENDEN

For symbols see § 7.8. $N = N_a \cdot N_b \cdot N_c$

Factors affecting covariance	Sum
	Basic formula
A	$N_b \cdot N_c \cdot {}^{N_a}S(\bar{x}_a - \bar{x})(\bar{y}_a - \bar{y})$
B	$N_c \cdot N_a \cdot {}^{N_b}S(\bar{x}_b - \bar{x})(\bar{y}_b - \bar{y})$
C	$N_a \cdot N_b \cdot {}^{N_c}S(\bar{x}_c - \bar{x})(\bar{y}_c - \bar{y})$
A and B	$N_c \cdot {}^{N_a \cdot N_b}S(\bar{x}_{ab} - \bar{x}_a - \bar{x}_b + \bar{x})(\bar{y}_{ab} - \bar{y}_a - \bar{y}_b + \bar{y})$
B and C	$N_a \cdot {}^{N_b \cdot N_c}S(\bar{x}_{bc} - \bar{x}_b - \bar{x}_c + \bar{x})(\bar{y}_{bc} - \bar{y}_b - \bar{y}_c + \bar{y})$
C and A	$N_b \cdot {}^{N_c \cdot N_a}S(\bar{x}_{ca} - \bar{x}_c - \bar{x}_a + \bar{x})(\bar{y}_{ca} - \bar{y}_c - \bar{y}_a + \bar{y})$
A, B and C	${}^{N}S(x - \bar{x}_{ab} - \bar{x}_{bc} - \bar{x}_{ca} + \bar{x}_a + \bar{x}_b + \bar{x}_c - \bar{x})(y - \bar{y}_{ab} - \bar{y}_{bc} - \bar{y}_{ca} + \bar{y}_a + \bar{y}_b + \bar{y}_c - \bar{y})$
Total	${}^{N}S(x - \bar{x})(y - \bar{y})$

where R_x, R_y are symbols for lengthy expressions whose form is obvious. When these two equations are multiplied together and summed, the cross-products of non-corresponding terms sum up to zero ; the proof is analogous to that given in a footnote in § 7.3. Moreover :

$$N_b.N_c.{}^{N_a}S(\bar{x}_a - \bar{x})(\bar{y}_a - \bar{y}) = N_b.N_c.{}^{N_a}S(\bar{x}_a\bar{y}_a) - N\bar{x}\bar{y}$$
$$= K_a - K_1$$

and so on. It will be found that the sums of products and degrees of freedom are as given in Table 12, which is analogous to Table 6.

The sums of products may, however, be negative.

To test the significance of the correlation which arises between x and y when the effects of factors B and C are averaged out, the effect of A remaining, the covariance k_a is formed by dividing the relevant sum of products by the number of degrees of freedom, thus :

$$k_a = \frac{K_a - K_1}{N_a - 1}.$$

The corresponding standard deviations s_{xa} and s_{ya} are found as in Table 6, whence for the interclass correlation coefficient r_a, § 4.4 :

$$r_a = \frac{k_a}{s_{xa} \cdot s_{ya}}$$

and the significance can be tested as in § 6.27. The other correlations can be treated similarly.

ARIATES x, y, AND THREE BALANCED FACTORS, A, B, C.

$S_{abc}=[xy]$

oducts	Mnemonic for signs and suffixes of K and N	Degrees of freedom
Formula for computation		
K_a-K_1	$a-1$	N_a-1
K_b-K_1	$b-1$	N_b-1
K_c-K_1	$c-1$	N_c-1
$K_{ab}-K_a-K_b+K_1$	$ab-a-b+1=(a-1)(b-1)$	$(N_a-1)(N_b-1)$
$K_{bc}-K_b-K_c+K_1$	$bc-b-c+1=(b-1)(c-1)$	$(N_b-1)(N_c-1)$
$K_{ca}-K_c-K_a+K_1$	$ca-c-a+1=(c-1)(a-1)$	$(N_c-1)(N_a-1)$
$K_{abc}-K_{ab}-K_{bc}-K_{ca}+K_a+K_b+K_c-K_1$	$abc-ab-bc-ca+a+b+c-1=(a-1)(b-1)(c-1)$	$(N_a-1)(N_b-1)(N_c-1)$
$K_{abc}-K_1$	$abc-1$	$N_a \cdot N_b \cdot N_c-1$

In this way it is possible to find out how far any correlation between x and y is attributable to the common effects of any factor or combination of factors. The interaction of A, B and C represents any correlation which has not been imposed by the main effects and first-order interactions of the balanced factors A, B, C; it includes, in addition to the second-order interaction (presumed negligible) and the effect of error, that of any direct causal relation between x and y. The significance of the differences between the second-order coefficient r_{abc} and other coefficients such as r_a can be tested as in § 6.27.

7.83 *Concomitant measurements.* Corresponding to each observation y let there be a value x of a factor which can be measured but not regulated. (For example, if y is the rate of growth of any child in a feeding experiment, x might be the original weight of the child.) If y is expressed as a percentage or fraction of x (e.g. if gain in weight is expressed as a percentage of the initial weight, or if a dose of a drug is divided by the body-weight of the recipient) the tacit assumption that y is proportional to x is made a priori and may not be true. The following method avoids this assumption.

In a two-factor experiment, § 7.3, with concomitant measurements x write:

$$\text{X for } {}^{N}S(x-\bar{x})^2 \text{ and}$$
$$\text{K for } {}^{N}S(x-x)(y-\bar{y}).$$

f then a linear regression can be formed for the whole of the observations it has, by § 4.2, the form:

$$Y-\bar{y}=\frac{K}{X}(x-\bar{x}).$$

TABLE 13. ANALYSIS OF VARIANCE OF A VARIATE CORRECTED FOR THE EFFECT OF CONCOMITA

$z = \frac{1}{2}\log_e(s^2a/s$
$n_1 = N_a - 1$
$n_2 = N - N_a - N$

Effect	Sum of products		Sum of squares for concomitant measurements x		Sum of squa
	Symbol	Formula, § 7.82	Symbol	Formula, § 7.83	Crude, § 7·3
A . . .	—	$K_a - K_1$	—	$X_a - X_1$	$S_a - S_1$
Error AB .	K_e	$K_{ab} - K_a - K_b + K_1$	X_e	$X_{ab} - X_a - X_b + X_1$	$S_{ab} - S_a - S_b + S_1$
A+AB . .	K_{ea}	$K_{ab} - K_b$	X_{ea}	$X_{ab} - X_b$	$S_{ab} - S_b$

Now $(Y - \bar{y})$ is that part of the value of an observation y which must be attributed to the effect of x; i.e. to the fact that the concomitant x differs from the mean concomitant $\bar{x}$ (e.g. that the initial weight of the specimen differs from the mean initial weight in the sample). Hence for the corrected value y'' of y obtained by eliminating the part in question :

$$y - y'' = Y - \bar{y} = \frac{K}{X}(x - \bar{x}).$$

$$\therefore\ y'' - \bar{y} = (y - \bar{y}) - \frac{K}{X}(x - \bar{x})$$

When the latter equation is squared and summed, it will be found that :

$$^{N}S(y'' - \bar{y})^2 = {}^{N}S(y - \bar{y})^2 - \frac{K^2}{X}$$

so that the total sum $^{N}S(y - \bar{y})^2$ can be corrected for the concomitant measurements by deducting K^2/X from it. At the same time the total degrees of freedom are diminished by 1 on account of the regression coefficient K/X. A similar process can be applied to any of the parts in an analysis of variance, and Fisher's method of eliminating the effect of the concomitant measurements from the test of significance is : (i) to apply the process to the error term—e.g. the interaction AB in § 7.3 ; (ii) to apply it to the error term plus the effect under test—e.g. AB plus A in § 7.3 ; and (iii) to base a z test on the resulting analysis of variance, Table 13.

This procedure is of general application, provided that the sums X and K always occupy corresponding positions in the tables of variance and covariance, Tables 6 and 12. If a linear regression is not obtainable, the variates x and y should be suitably transformed, § 4.24.

EPENDENT UPON TWO BALANCED FACTORS A, B,

EASUREMENTS x, §§ 7.3, 7.82, 7.83

r dependent variate y	Degrees of freedom		Corrected sum of squares ÷ corrected degrees of freedom
Corrected, § 7.83	Crude, § 7.3	Corrected, § 7.83	
$S_a - S_1 - \frac{K^2_{ea}}{X_{ea}} + \frac{K^2_e}{X_e}$	$N_a - 1$	$N_a - 1$	s^2_a
$S_{ab} - S_a - S_b + S_1 - \frac{K^2_e}{X_e}$	$(N_a - 1)(N_b - 1)$	$(N_a - 1)(N_b - 1) - 1 = N - N_a - N_b$	$s_e{}^2$
$S_{ab} - S_b - \frac{K^2_{ea}}{X_{ea}}$	$N_b(N_a - 1)$	$N_b(N_a - 1) - 1 = N - N_b - 1$	—

In Table 13 the following symbols have meanings analogous to those of the symbols S for y, § 7.3 :

$$X_a = {}^{N_a}S(\bar{x}_a \,.\, [x_a]) \; ; \; X_b = {}^{N_b}S(\bar{x}_b \,.\, [x_b]) \; ;$$
$$X_1 = \bar{x}[x] \; ; \; X_{ab} = {}^{N}S(x^2).$$

7.9 **Missing observations**

One or more observations may be missing from the complete set envisaged by the factorial design of an experiment. For instance, one or more combinations of factors may be precluded by the pain rule attached to experimental licences, or one or more animals may develop painful symptoms and have consequently to be killed before the conclusion of the experiment. Fisher's method of restoring the balance is to write an algebraic symbol such as y_1, y_2, y_3 . . . in the place of each missing observation in a table such as Table 5, and to find an appropriate value for it by the rule of least squares.

Let S_e be the sum of squares for error ; it will be a quadratic function of the symbols y. In a one-factor experiment it will be $(S_{ab} - S_a)$, equation (7.23c), and will consist partly of squares of the actual observations and their means, and partly of algebraic terms that arise through including y_1, y_2 . . . among the observations. Then the rule of least squares gives a set of simultaneous linear equations :

$$\frac{\partial S_e}{\partial y_1} = 0, \; \frac{\partial S_e}{\partial y_2} = 0, \; \ldots$$

and so on, whence the value of each y can be calculated, and hence the arithmetical value S'_e of S_e. The number of degrees of freedom will be equal to that which would have obtained if the experiment had been complete, less the

number of missing observations; i.e. it is based on the actual number of observations.

For example, if there is only one missing observation, denoted by y_1, and if

$$S_e = ay_1^2 - 2by_1 + c$$

where a, b and c are arithmetical quantities computed from the observations:

$$0 = \frac{\partial S_e}{\partial y_1} = 2ay_1 - 2b$$
$$y_1 = b/a$$
$$S' = c - b^2/a.$$

To test the significance of a factor A, a similar procedure is adopted for the term S_{ae} comprising the sum of squares for error plus that for A. Thus if

$$S_{ea} = S_e + S_a - S_1$$

where each of these terms is a quadratic function of the missing values y, then write:

$$\frac{\partial S_{ea}}{\partial y_1} = 0, \quad \frac{\partial S_{ea}}{\partial y_2} = 0, \quad \ldots$$

From the resulting values of the symbols y a purely arithmetical sum S'_{ea} is calculated, with degrees of freedom based, like those of S'_e, on the number of actual observations. Then the adjusted sum of squares for A is $(S'_{ea} - S')$ with degrees of freedom equal to those in the complete experiment as originally designed. The values of $y_1, y_2, \ldots$ in the two cases S'_{ea} and S'_e will be slightly different; the difference allows for the sampling error of the adjustment.

8. Biological assay

The potency of manufactured lots of newly developed pharmaceutical products, such as vitamins and hormones, is usually assayed by comparing the biological effect of a sample with that of a standard preparation. The error of such an assay is usually large, and on every ground it is desirable that as soon as a valid chemical or physical test can be devised it shall take the place of the biological test.

Such a biological test must be: (1) humane, (2) relevant, and (3) sufficiently precise. The third of these requirements has an abundant literature, of which summaries and bibliographies have been given by:

J. O. Irwin, *Suppl. J.R. Stat. Soc.*, **4**, 1 (1937).
C. I. Bliss and M. Cattell, *Ann. Rev. Physiol.*, **5**, 479 (1943).
D. J. Finney, paper read to R. Stat. Soc. 30 Jan. 1947.

Though the first of the three requirements has by no means been neglected it has yet to enjoy an equally thorough investigation. For instance: (1) in order to make assays more sensitive the animals are often prepared by techniques which involve inanition or other extreme conditions, or surgical interference. It is desirable to improve the statistical and metrical technique so far that the requisite precision may be obtainable with more normal animals:

(2) When the test reaction is death there need be no question of inhumaneness if the fatal symptoms are painless, as in testing narcotics, or if anæsthesia is used, as in assaying digitalis by progressive dosage ; but on the other hand there are other techniques which call for an endeavour to substitute the measurement of some painless graded response for the percentage mortality. Moreover the dose LD50, which requires the fewest observations, also produces the most prolonged symptoms. When, therefore, in spite of objections, a decision to use such a method has been taken, the question arises whether it is better to work with relatively few animals in the middle of the probit range or to work with more animals near the ends of the range so that mild symptoms will occur at one end and a quick death at the other. In any case, animals which are obviously dying might be killed.

By *relevance* is meant the relation of the assay, and especially its precision, to the ultimate purpose for which it is made. When a lot which is to be used as a standard is being assayed against an existing standard, the precision must be high if cumulative errors are to be avoided ; but when a drug is to be sold for use in medical practice it is necessary to know how the test reaction used in the assay is correlated with the effect that is to be produced in the human patient, for different species react differently to the same drug. No purpose other than a purely commercial one is served by making the precision of the assay greater than that with which the man-animal correlation has been ascertained, and usually the latter will be low in view of the variability of human response. If precision in the assay is swamped by error in the man-animal correlation it becomes superfluous.

The relevance or man-animal correlation cannot be experimentally ascertained when the purpose of the test is to specify the risk of a dose being fatal to man, and the use of assays for this purpose involves extrapolation. It would seem, therefore, that a mortality test has no advantage, in such cases, over any other test which distinguishes levels of potency, and the substitution of some innocuous graded response would appear to be preferable.

Percentage mortality is also used for assaying curative potency, and in this case the man-animal correlation can in theory be ascertained by experiment. A priori such correlations must be expected, however, to have large experimental errors, for one end of the human response/dosage regression is being compared with the opposite end of the corresponding animal regression. It would appear to be better to form the correlation between analogous reactions in man and animal. In any case it is medically useless to make the assay much more precise than the man-animal correlation or relevance.

9. Planning

9.1 The over-riding consideration

Animal biology differs from other experimental research in that it deals with sentient material and is consequently subject to ethical restrictions. Experiments on human beings which are likely to be fatal and experiments on animals which are likely to cause serious suffering must be omitted. This is the over-riding consideration in planning any experiment.

9.2 **Precision**

The experimenter must decide on the level of significance to which he is to work. If this is too severe, then either important results will too frequently be rejected, or an unnecessarily large number of observations will have to be made; but if it is too lenient the experimenter will relatively often follow a false scent. In either case animals will be wastefully used. A common working rule is that the null hypothesis should be rejected or accepted if the fiducial probability P is definitely below or above 0·05, while if P is close to 0·05 more observations should be made and should be combined with those already obtained, § 5.

For high precision with few observations the error term in a test of significance must be made as small as possible. To this end each block, § 7, should comprise observations on animals from the same litter and sex, and inbred homogeneous stock should be used; but to widen the inductive basis of the experiment different blocks should be drawn from more than one such stock of animals. Each block is treated as one phase of a regulated factor, § 7.

Fig. 70 shows, as a multiple of the estimated standard deviation s, the numerical range of error $\pm\delta$ in estimating a mean from N observations. This range is calculated as follows for three different levels of significance α:

$$\pm\delta = \frac{st'}{\sqrt{N}}$$

where the value of t' is found from the table of Student's t for $(N-1)$ degrees of freedom, against a probability P equal to α. It will be seen that even when homogeneous stock is used, so that s is substantially constant, the accuracy increases but slowly as the sample-size is increased after the first half-dozen observations. If, however, the sample-size is increased at the expense of homogeneity—if, for instance, purchased animals are added to homogeneous stock—then the increase in sample-size is likely to lead to a loss of precision.

9.3 **Economy in sampling**

There is no such thing as absolute accuracy in experiment, and the use of hundreds of animals in a blind quest for precision is mere blundering in the dark; it may even lead to a decrease of precision through an increase in heterogeneity. Precision must bear a considered relation to the object of the investigation and to the experimental conditions.

It is advantageous to design an experiment so that if the result is inconclusive it may nevertheless be utilized in combination with the results of subsequent replications. For instance, if a small-scale Latin-square design yields a result on the borderline of significance that result can, if the experiment is well planned, be combined with those of subsequent repetitions based on cyclically related Latin squares.

Alternatively waste may be avoided by forecasting correctly the number of animals required to obtain a result which is just conclusive. A. H. J. Baines has developed a method which offers great promise (typescript QC/R/15 of the

Ministry of Supply's Advisory Service on Statistical Method). His procedure is to : (1) decide upon a level α of significance ; (2) decide upon a margin for negligible error in the null hypothesis (e.g. if the null hypothesis is that a population mean is zero, and if a margin $\partial\bar{\xi}$ is decided upon, then the population mean shall be considered to be zero if its true value is less than $\partial\bar{\xi}$) ; (3) decide upon a fraction β such that the chance of wrongfully accepting the null hypothesis (e.g. of failing to detect a true value of the population mean if this is in fact greater than $\partial\bar{\xi}$) must not exceed β when the level of significance is α ; and (4) design the experiment so as to secure a just sufficient assurance, as defined by α and β, of detecting a divergence of the null hypothesis from truth when that divergence exceeds the prescribed margin $\partial\bar{\xi}$.

In the instance cited, if the variance σ^2 of a homogeneous population with normally distributed error is approximately known, the requisite number N of observations is forecast thus :

$$N=\left(\frac{\sigma}{\partial\bar{\xi}}\right)^2\{\text{probit}\,(\alpha/2)+\text{probit}\,\beta-10\}$$
$$=2\sigma^2/(\partial\bar{\xi})^2 \text{ when } \alpha=0{\cdot}05 \text{ and } \beta=\tfrac{1}{2}.$$

For factorial designs it is necessary to specify a margin Q for $\sigma_A{}^2/\sigma_e{}^2$, § 7.2. Then

$$N_b\,.\,Q+1=F_\alpha/F_{(1-\beta)} \quad . \quad . \quad . \quad . \quad . \quad . \quad (9.3A)$$

where F has the distribution referred to in § 3.35 and its subscripts are values of P, so that when α and β have been chosen equation (9.3A) gives a relation between N_b, Q and N_a, the latter being implicit in F. Then the minimum value of N can be found as the minimum of $N_a\,.\,N_b$. The theory awaits experience in its application to biological research.

When the object of an experiment is to form an estimate p of the fraction of a population which exhibits a given attribute, and to do so with a standard error ϵp (e.g. to form an estimate $100p$ of the percentage of blood corpuscles which belong to a given type, and to have a standard error of 100ϵ per cent in p) and if ϵ can be taken as approximately constant during variations of p, J. B. S. Haldane recommends (*Nature*, 13 January, 1945) that the experiment shall proceed until m individuals with the attribute in question have been found, where

$$m=2+(1-p)/\epsilon^2.$$

Then

$$p=\frac{m-1}{N-1}.$$

Now m can be forecast, for either p may be known approximately in advance, or it may be small, or it can be computed at intervals as the experiment proceeds. There may be instances in which this method could be applied to mortality tests or the like.

This is an independent example of what is called *sequential analysis* or *serial sampling*, which was developed during the late war and is now being released from secrecy—sequentially. Further examples will be found in current and forthcoming parts of the *Journal of the Royal Statistical Society*,

and in the O.S.R.D.'s *Sequential Analysis of Statistical Data* (Oxford University Press, 42*s*.), reviewed in *Nature*, 18 May, 1946.

9.4 **Design**

If the object of the experiment is to ascertain whether or not several conditions have significant effects, a factorial experiment with factors at two levels, §§ 7.5, 7.7, will give the required information. If, on the other hand, a quantitative relation between factor and response is required, a preliminary two-level experiment will show which factors are worth considering and a factorial square or randomized block, § 7, can then be planned. The design should be such that the results obtained from repetitions can be combined, § 5, so that if the first test is inconclusive it shall not be wasted. The requisite number of observations should be forecast with due regard to economy of sampling, § 9.3.

Any variations such as initial weights or other factors which can be measured but not regulated should be recorded and allowed for as in § 7.83.

If any observations are missing, as a result for instance of animals having to be put out of pain before the conclusion of the experiment, the balance must be restored by the calculation referred to in § 7.9.

The time to consult a statistician is before, not after, the experiment.

10. Literature

It is hoped that this appendix will afford a key to the detailed treatment available in periodicals and text-books, which give numerical examples worked out in full. The differences between the notations used in different publications should be borne in mind ; for instance, Tippett uses n, N, where Mather uses N, n, Fisher n, n', and Kendall ν, n. The text-books contain excellent bibliographies, and Yule and Kendall in particular give an extensive classified list of references. Additional references are published periodically in the journals of the Royal Statistical Society and the American Statistical Association.

Industrial Statistics by H. A. Freeman (Chapman & Hall, 1942, 15*s*.) gives a lucid introduction to the principles of statistics and contains some useful tables not readily available elsewhere.

Statistical Analysis in Biology, by K. Mather (Methuen, 1943, 16*s*.) and *The Methods of Statistics* by H. C. Tippett (Williams & Norgate, 1941, 17*s*. 6*d*.) may advantageously be read together. Each treats some points more clearly or fully than the other.

An Introduction to the Theory of Statistics by G. Udny Yule and M. G. Kendall (Griffin & Co., 1940, 24*s*.), written from the classical point of view but containing much modern theory, goes thoroughly into the logic of the subject.

Statistical Methods for Research Workers, by R. A. Fisher (Oliver & Boyd, 1942, 16*s*.), and *The Design of Experiments*, by R. A. Fisher (Oliver & Boyd, 1944, 12*s*. 6*d*.) are treasuries of information written by the founder of the modern theories of small samples and design of experiments. They are the statistical Koran of the biologist and make equally exacting demands on the devout reader.

Statistical Mathematics by A. C. Aitken (Oliver and Boyd, 1942, 4*s.* 6*d.*), gives the mathematical theory in an admirably condensed form.

The Advanced Theory of Statistics by M. G. Kendall (Griffin & Co., vol. 1, 1943, 42*s.* ; vol. 2, 1946, 50*s.*) is a comprehensive treatise addressed to mathematicians but is so lucidly written as to be surprisingly readable.

Statistical Tables for Biological, Agricultural and Medical Research by R. A. Fisher and F. Yates (Oliver & Boyd, 1943, 13*s.* 6*d.*), has an informative introduction explaining the use of the tables.

A discussion of the *Statistical Treatment of Animal Experiments*, opened by Dr. John Wishart and continued by R. A. Fisher, F. Yates, Miss Turner, S. Bartlett, M. S. Bartlett and F. J. Dudley is published in *J. R. Stat. Soc., Suppl.*, vol. 6, no. 1.

Graph paper in various logarithmic and probability rulings can be obtained from Wightman Mountain, Ltd., Artillery Row, London, S.W.1.

11. Envoi

The writer's grateful thanks are due to Mr. A. H. J. Baines for resolving many puzzles for him, and for constructive criticisms of the first draft of this Appendix.

Among the innovations that have been introduced may be mentioned the notation for the analysis of variance and covariance, with the resulting relation set out in § 7.25 and Tables 6 and 12, and the graphical tests of significance, Figs. 66 to 69. The latter are rough ; but then levels of significance are arbitrarily chosen.

The Appendix is intended to provide a compendium of principles and formulæ useful to biologists, and to serve this further purpose. The elementary text-books are written by expert statisticians, who sometimes take for granted things that are enigmas to the beginner. The present writer has tried to furnish answers to all the questions that puzzled him when he recently read up the subject for the first time, and to express concepts and principles in words that will be understood by the intelligent inexpert if the latter will construe them rigorously.

The writer will not have burned his midnight oil in vain if as a result even one rat the fewer shall be required in some experiment involving discomfort.

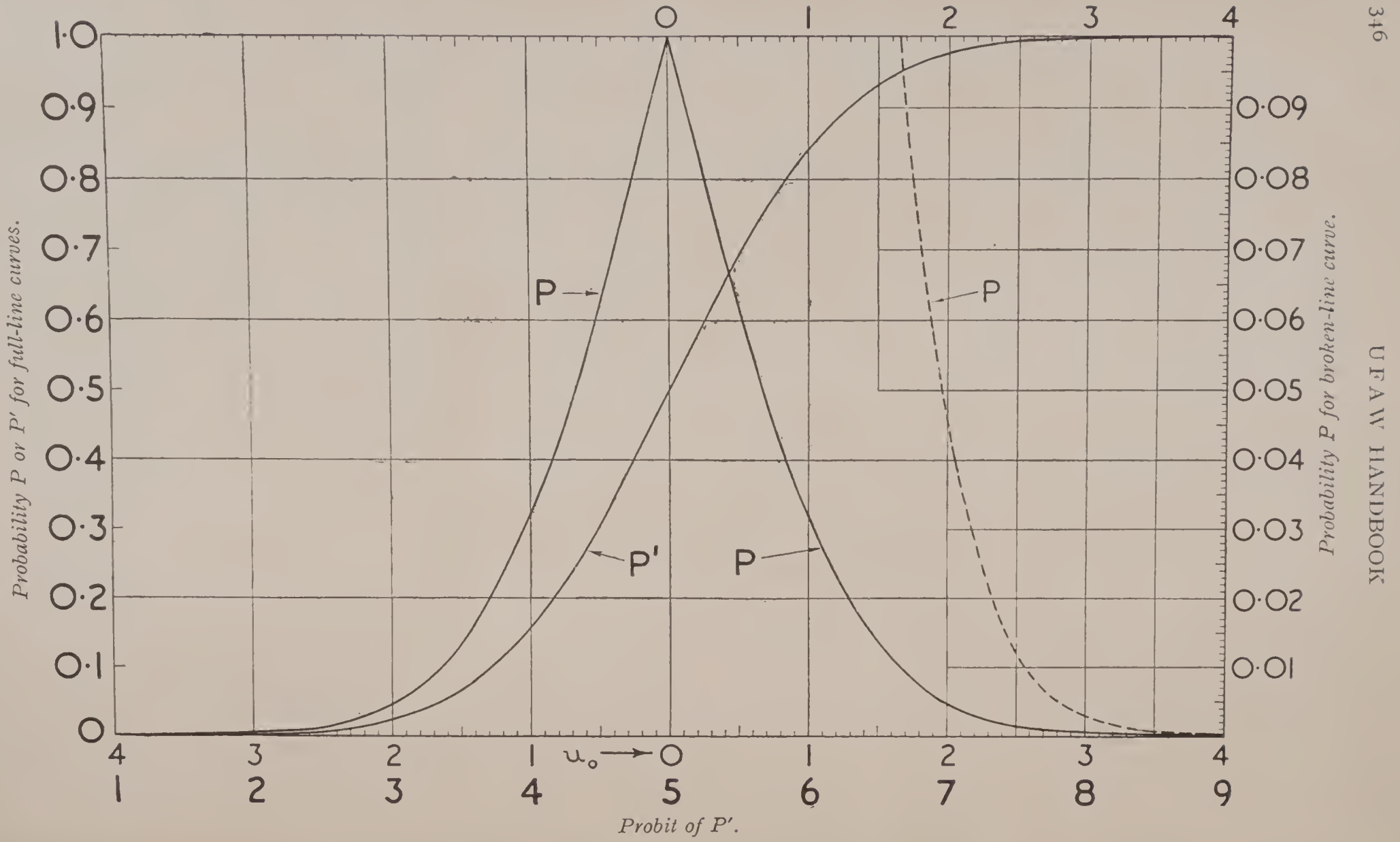


FIG. 65. THE STANDARD NORMAL TEST OF SIGNIFICANCE; PROBITS.

P is the probability that a random value of u shall be numerically greater than u_0, while P′ is the probability that it shall be

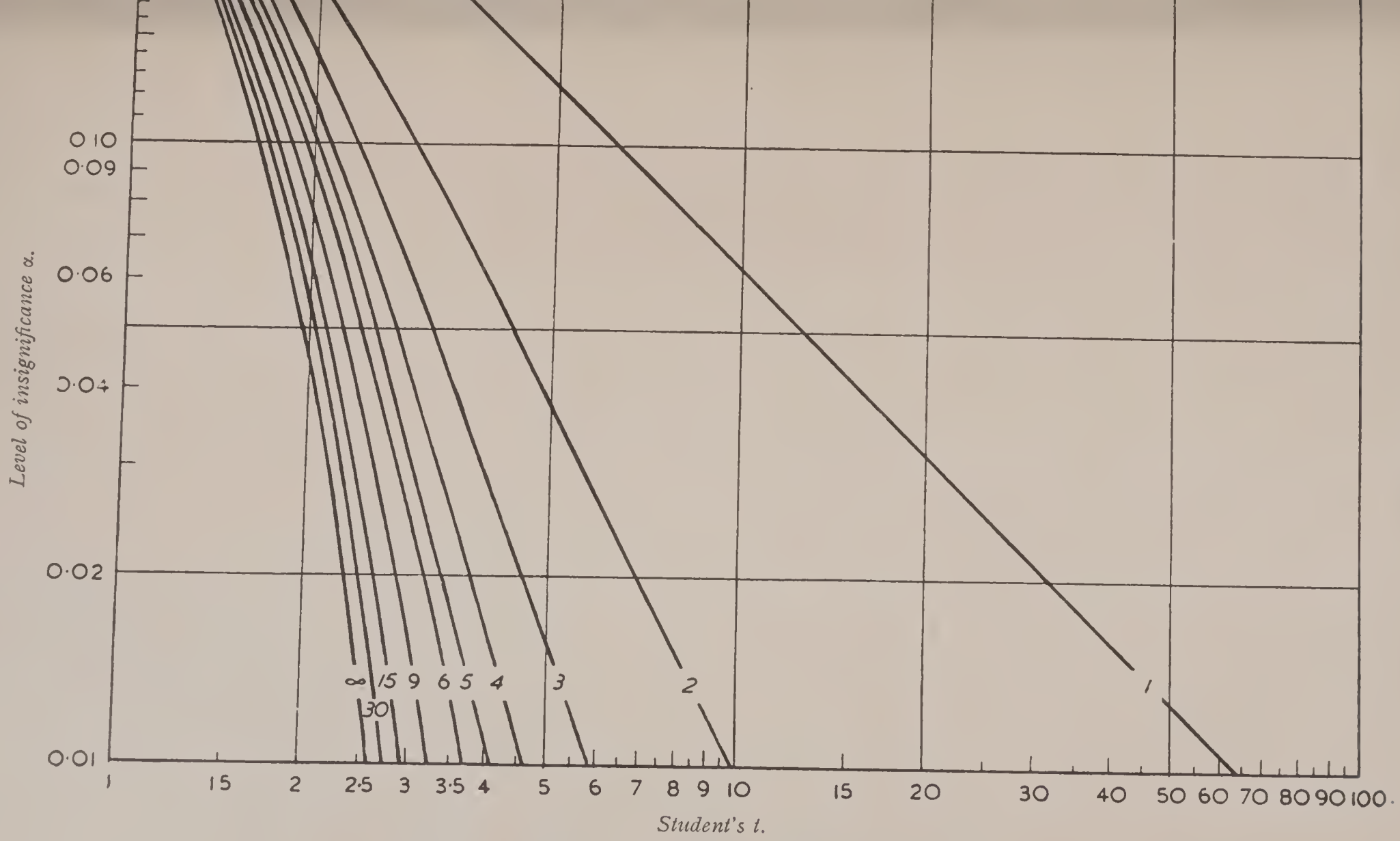


FIG. 66. TESTING FOR SIGNIFICANCE WITH STUDENT'S *t*.

Lay a sheet of paper with its top edge horizontal along the chosen level of significance α and its right-hand edge passing through the experimental value of *t*. The null hypothesis is rejected if the top right-hand corner of the sheet lies to the right of the graph corresponding to the number *n* of degrees of freedom marked on the graph.

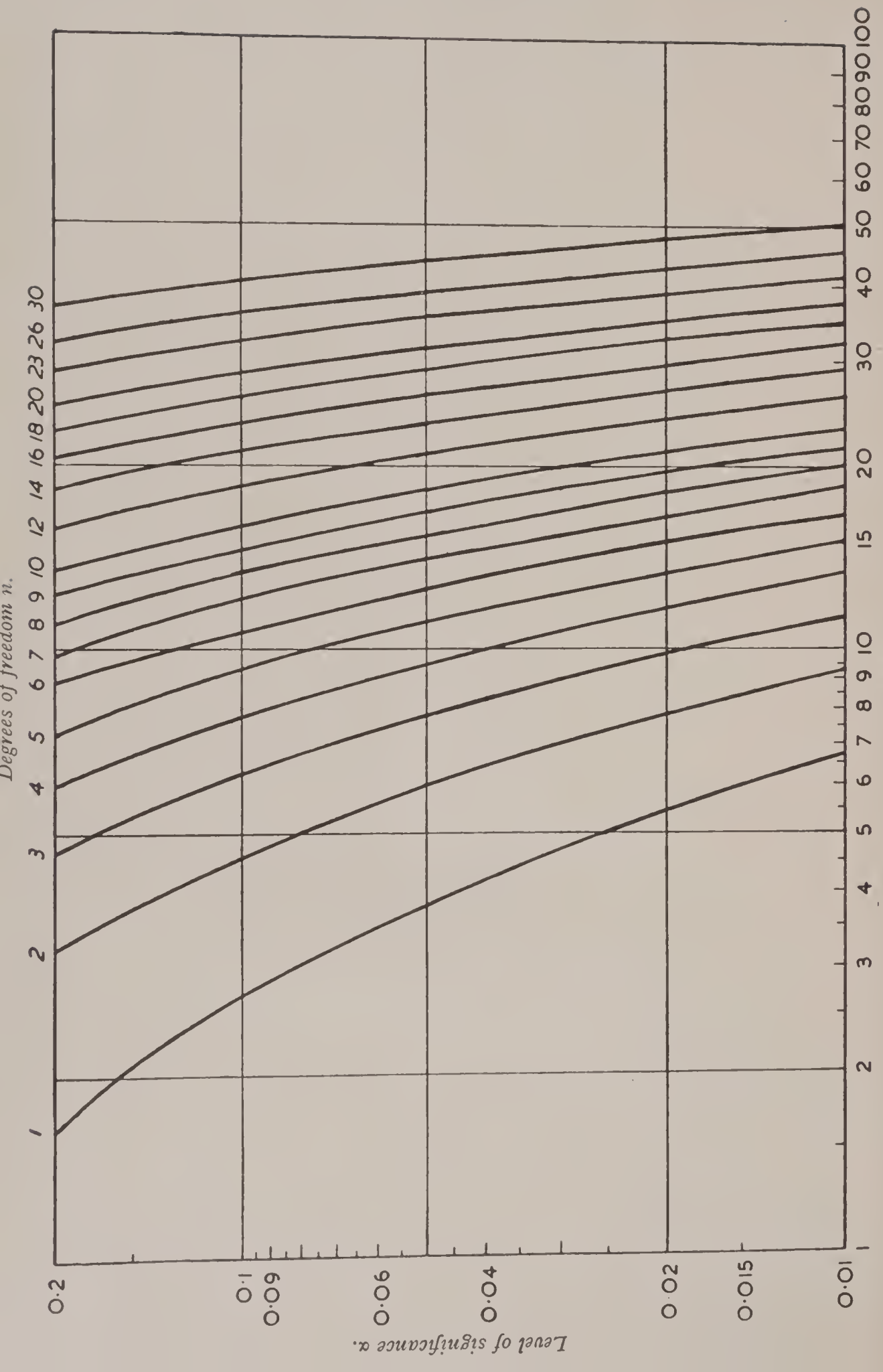


FIG. 67. TESTING FOR SIGNIFICANCE WITH THE SQUARE CONTINGENCY χ^2.

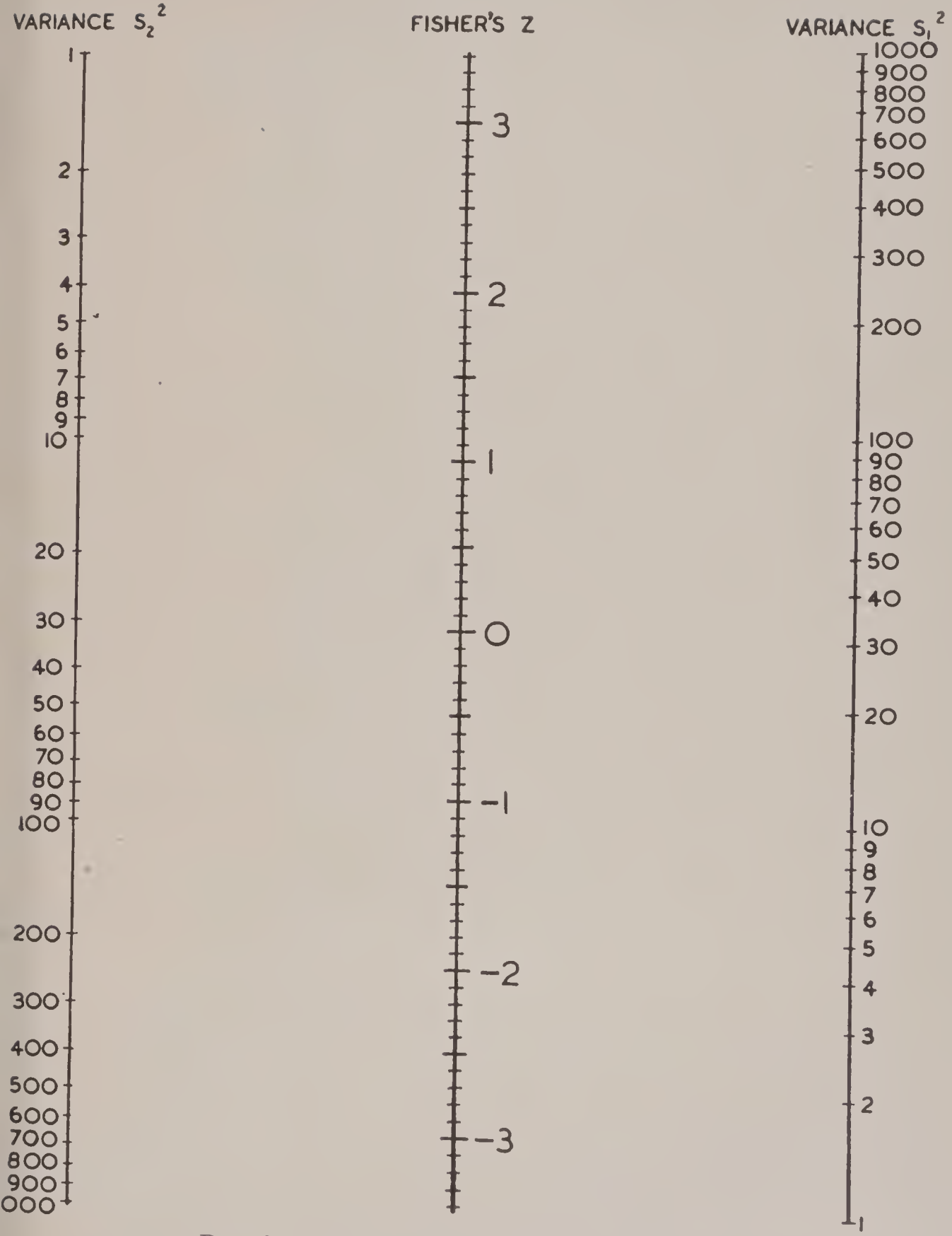


FIG. 68. NOMOGRAM FOR COMPUTING FISHER'S z.

Lay a straight edge, such as that of a sheet of paper, so that it passes through the value of s_1^2 on the right-hand scale and through that of s_2^2 on the left-hand scale. Then it passes through the value of z on the middle scale.

If necessary multiply or divide s_1^2 and/or s_2^2 by any power of 10. If as a result s_1^2/s_2^2 is multiplied (or divided) by 10^m then add $1{\cdot}151m$ to (or subtract it from) the scale reading of z.

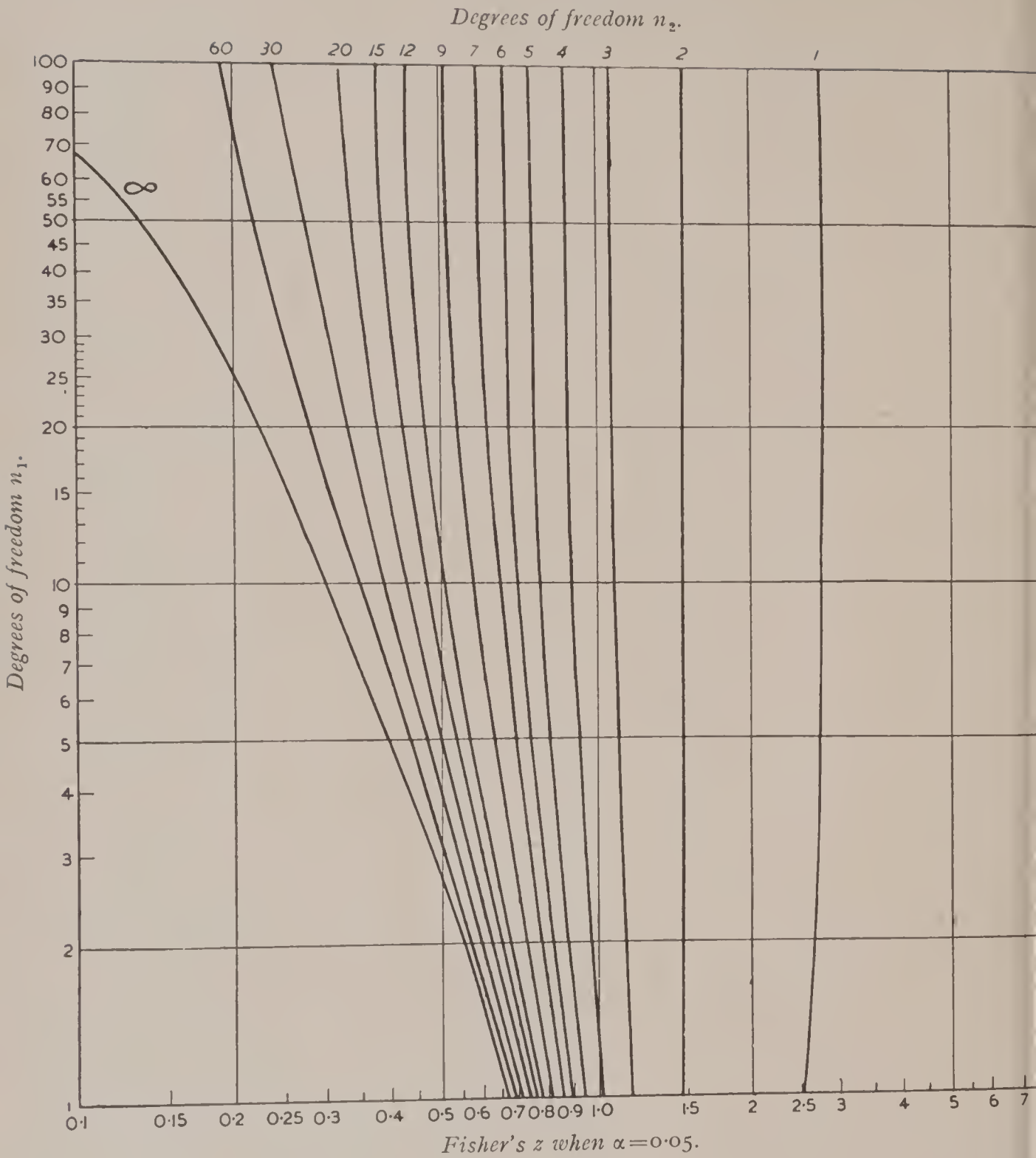
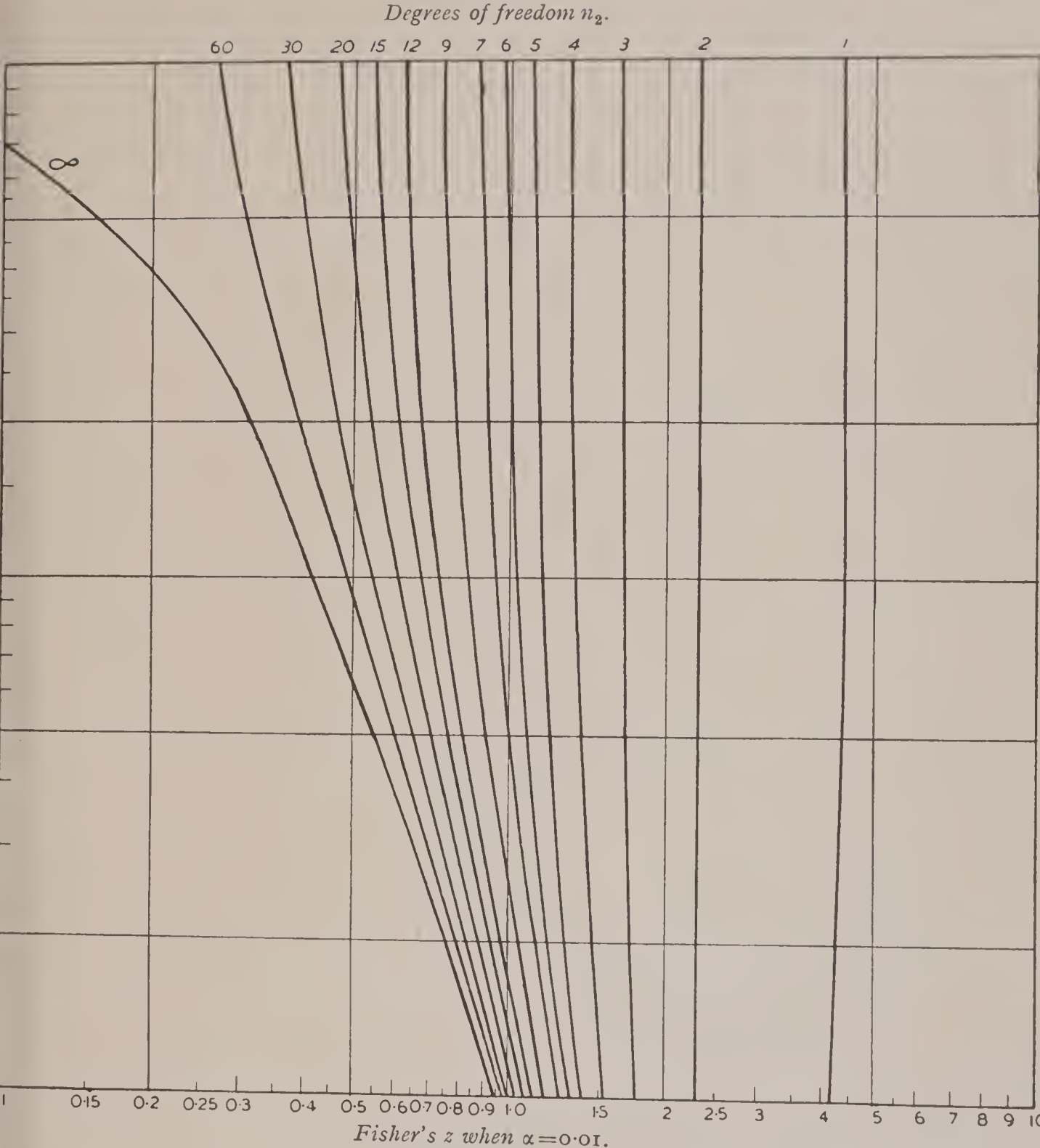


FIG. 69 (A). TESTING FOR SIGNIFICANCE WITH FISHER'S z WHEN THE LEVEL OF SIGNIFICA IS 0·05.

Lay a sheet of paper with its top edge horizontal and passing through the value of n_1 on the scale, and with its right-hand edge passing through the value of z on the horizontal scale. the null hypothesis if the top right-hand corner of the paper lies to the right of the graph corres to n_2.



69 (B). TESTING FOR SIGNIFICANCE WITH FISHER'S z WHEN THE LEVEL OF SIGNIFICANCE IS 0·01.

a sheet of paper with its top edge horizontal and passing through the value of n_1 on the vertical
nd with its right-hand edge passing through the value of z on the horizontal scale. Reject the
pothesis if the top right-hand corner of the paper lies to the right of the graph corresponding to n_2.

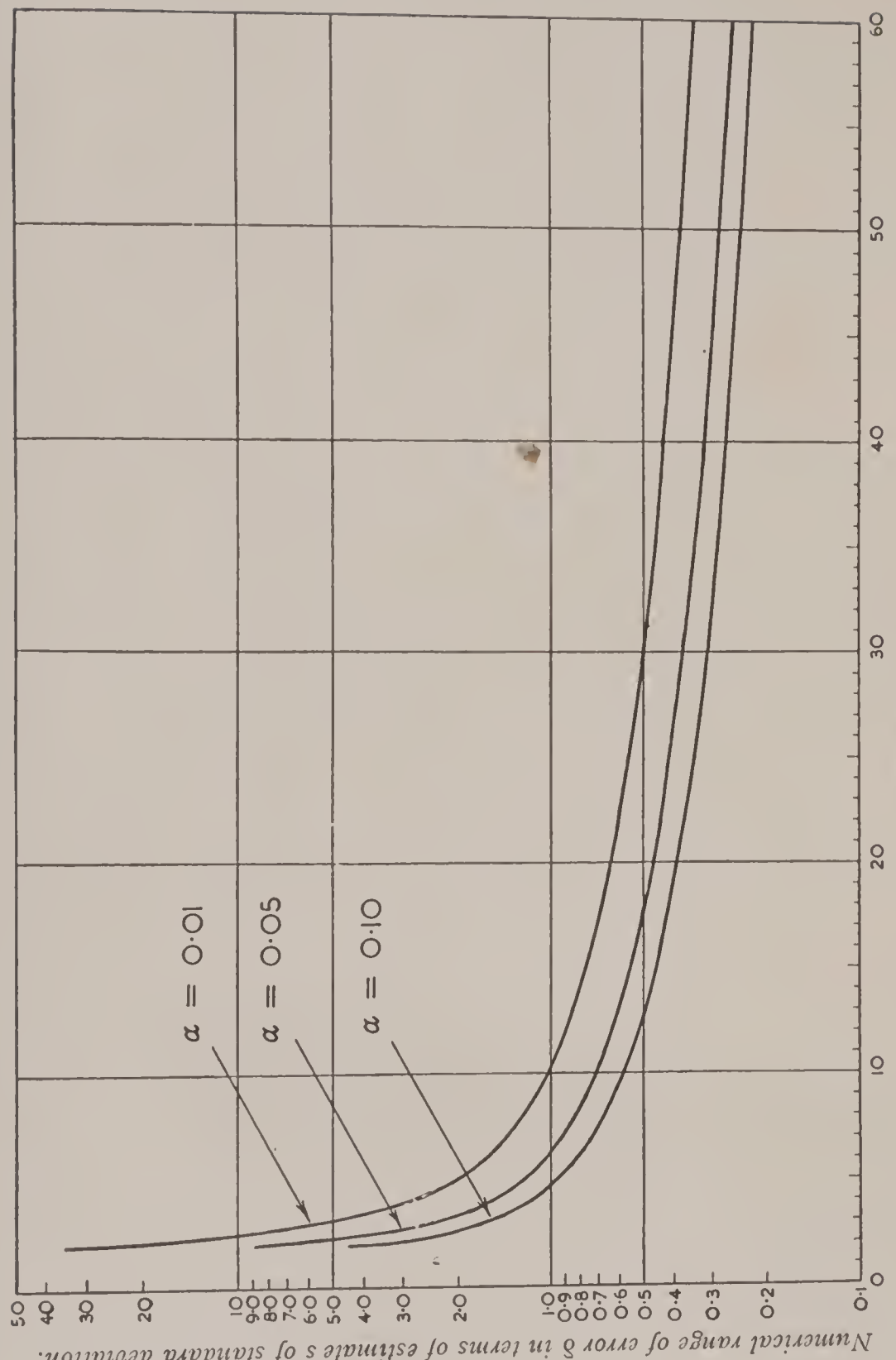


FIG. 70. EXPERIMENTAL ERROR AS A FUNCTION OF SAMPLE-SIZE.

The ordinate shows, as a multiple of the estimate s of the standard deviation, the error in estimating, from a sample, the mean of a population. The chances are 0·01, 0·05 and 0·1 respectively that the estimated mean shall be in error by more than s times the figures read from the graphs against the number N of observations in the sample.

If an increase in the sample-size N involves an increase in s (e.g. if purchased animals are added to homogeneous stock to make up a larger sample) the error will be increased proportionately, and may be greater

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